PHD

An investigation of the cardiovascular effects of the chronic administration of atenolol and nitrendipine given alone and in combination

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AN INVESTIGATION OF THE CARDIOVASCULAR EFFECTS OF THE CHRONIC ADMINISTRATION OF ATENOLOL AND NITRENDIPINE GIVEN ALONE AND IN COMBINATION.

submitted by Martyn Pearce Kingsbury

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To my family.

"Discovery consists of seeing what everybody has seen and thinking what nobody has thought."

.... Albert Szent-Gyorgi,

(The Scientist Speculates, 1962, ed Good, I., London, Heineman)

SUMMARY.

1. The aim of this work was to investigate any interaction(s) between the β-adrenoceptor blocker atenolol and the calcium antagonist nitrendipine, following chronic administration, using *in-situ* methods where possible. Blood pressure and heart rate were investigated in conscious normotensive and hypertensive rats, followed by examination of adrenergic neurotransmission in the mesenteric vascular bed in both rats and dogs.

2. Work has shown that daily oral administration of atenolol administered alone or in combination produces a significant degree of β-blockade, and a reduction of heart rate and blood pressure in the conscious normotensive and hypertensive rat. Work with hypertensive rats, and normotensive rats and dogs, indicated that following chronic treatment atenolol had some presynaptic effect. This conclusion was supported by the examination of stimulus induced ^{3}H-noradrenaline overflow. The size and time course of this effect suggests that it may be important in the reduction in blood pressure observed clinically.

3. Nitrendipine treatment has been shown to be effective in reducing blood pressure in normotensive and hypertensive rats. However, a reflex tachycardia was observed in normotensive animals. Nitrendipine treatment appeared to act post-synaptically to reduce mesenteric responses to both endogenous and exogenous noradrenaline.

4. Treatment with the combination of atenolol and nitrendipine reduced both blood pressure and heart rate in normotensive and hypertensive rats. There was, however, no evidence of an additive hypotensive effect. This may have been due to the complex changes in adrenergic neurotransmission observed with the combination. These suggested, that following chronic administration, non-noradrenergic neurotransmission was involved in maintaining responses to electrical stimulation. This could be the result of the "unmasking" of a co-transmitter following the profound reduction in response to noradrenaline. The interaction between atenolol and nitrendipine did, however, prevent the reflex tachycardia observed with nitrendipine alone.

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et seq.

CHAPTER 1.

INTRODUCTION

1.0 INTRODUCTION.

1.1 Aims of the investigation.

Two of the most important groups of antihypertensive drugs, β-adrenoceptor blockers and calcium antagonists, are increasingly used in combination. The rationale for their combined use is based on the theoretical consideration that these agents produce a similar hypotensive effect via their interaction at different sites of action. The clinical observation of their increased efficacy and a reduced incidence of side effects when given in combination supports this view. The pharmacological knowledge of the sites and mechanisms of action of these agents comes largely from the examination of one or other of the groups in isolation. Additionally, in the clinical control of hypertension, these drugs are likely to be administered for long periods of time, whereas the majority of the pharmacological investigations have examined the effects of only acute administration.

Previous work at the University of Bath has examined the effects of various β-adrenoceptor blocking drugs following chronic administration. One of the important considerations to come from this work is that the *in vitro* investigations can often be complicated by the "wash-out" of the β-blocker following tissue removal.

The initial aims of this present work were to examine the possible interactions between β-adrenoceptor blocking drugs and calcium antagonists following their chronic administration, and to investigate the mechanisms involved, using *in situ* experimental techniques where possible.

The investigation was undertaken using the β-adrenoceptor blocker atenolol and the calcium antagonist nitrendipine. The pharmacology of these two drugs is such that they appear particularly well suited for use in combination.

1.2 An introduction to the scheme of the investigation.

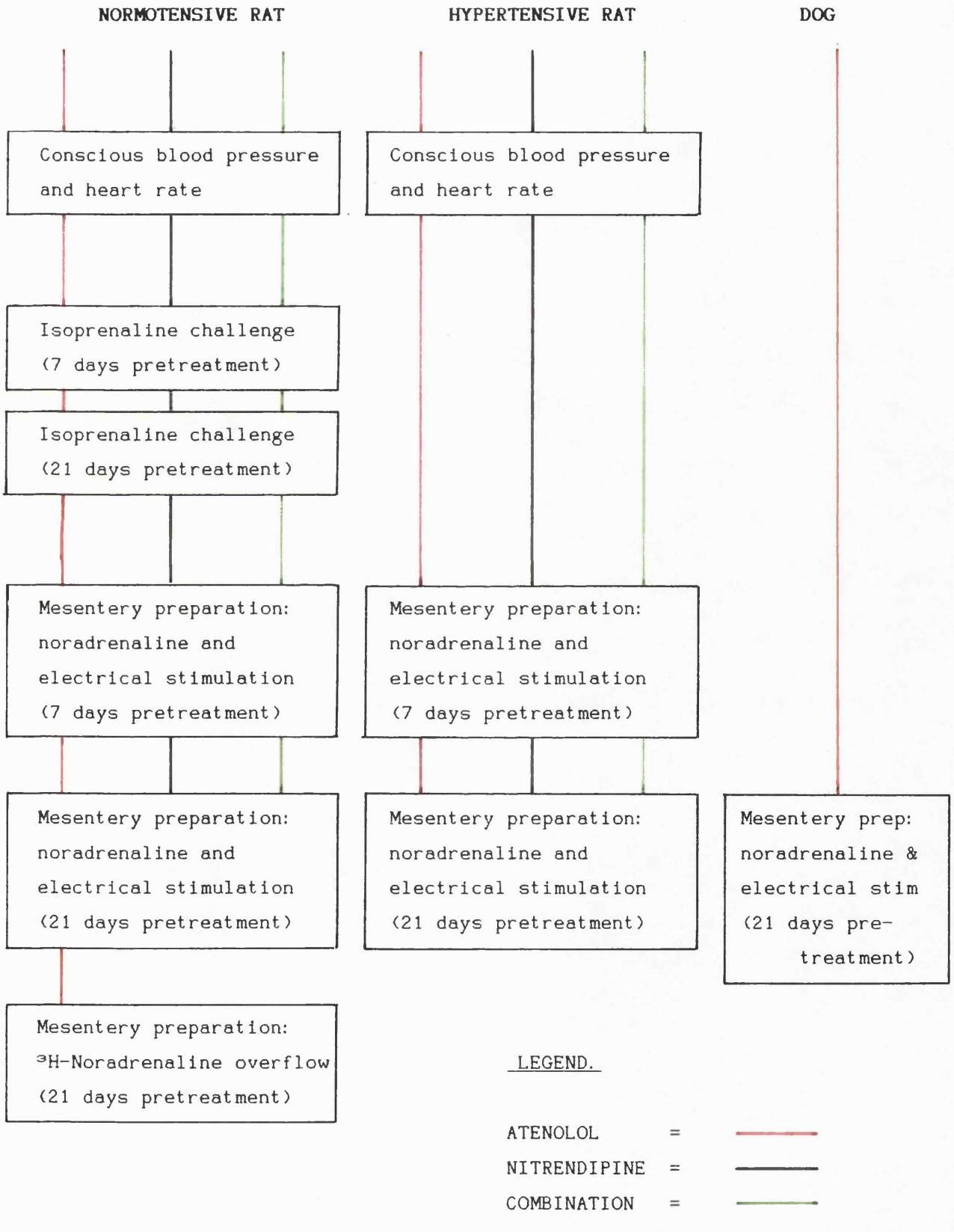
The first stage of the investigation was to examine the effects of the drugs on the blood pressure and heart rate of conscious rats over a three week period of daily administration.

The next stage was an investigation of the presence of β-adrenoceptor blockade by examining the response to a standard isoprenaline challenge. This was carried out after 7 and 21 days treatment.

After this, the effect of 7 and 21 days pretreatment on adrenergic neurotransmission was examined using an *in situ* mesentery preparation. Finally, this preparation was used in a preliminary examination of ^{3}H-noradrenaline overflow.

This plan was followed using normotensive and hypertensive rats and normotensive dogs, after pretreatment with atenolol and nitrendipine alone and in combination. The exact scheme followed is shown in the flow diagram overleaf.

FIGURE 1: A schematic diagram of the work undertaken in the investigation.



1.3 Introduction to the scheme of the thesis.

The schematic representation of the investigation, shown in figure 1, shows that the work can most easily be divided into three sections according to the three treatment regimes. In order to set out the information in a logical order the main body of this thesis is divided into three chapters, each corresponding to a treatment group. Each of these three chapters is further divided to include: introduction, methods, results and conclusions sections. This initial chapter aims to introduce the general items which do not fall into the three treatment chapters. The main body of the thesis is followed by a final chapter which brings together the conclusions from each of the treatment chapters and attempts to discuss them further and suggest future work.

1.4 Introduction to some general points of methodology.

1.4.1 Blood pressure measurement in conscious animals.

Arterial blood pressure is the hydrostatic pressure generated within the arterial system by the heart pumping blood against the peripheral resistance of the vessels. The direct measurement of blood pressure requires surgical cannulation of a major artery and subsequent connection to a recording system. While this approach provides the most accurate method of measurement and is ideal for anaesthetised acute work, it has several drawbacks. The main disadvantages for chronic work in conscious

animals are the preparation time involved and the problems in maintaining infection free postoperative animals with "unclogged" cannulas.

The indirect measurement of blood pressure allows repeated measurements in a large number of animals but is less accurate. Despite this somewhat reduced accuracy, reliable comparative measurements can be made if the experimental conditions are strictly controlled.

The indirect method of blood pressure measurement was chosen for use in this study. The exact method used follows the principle of occluding the blood flow to the tail with a pneumatic cuff and recording the pressure pulses in the caudal artery as the cuff pressure is increased and released. In order to be able to record the pulse pressure of the caudal artery, it has to be dilated, and to facilitate this the rats are pre-warmed at 39°C (Lovenberg 1987). Every effort is made to ensure the results are as accurate and reproducible as possible by: habituating the rats to the procedure, warming, and placing the cuff and probe consistently, and avoiding extraneous activity whilst recording. (for details see section 2.2.1) Despite every effort, animals can become stressed by the warming and this may increase the variability of the results. This can be avoided to some extent by the latest probes which use photoelectric rather than pressure sensors. Such photoelectric probes can detect pulsatile flow without the necessity of dilating the caudal artery, and thus negate the need for warming. Unfortunately such a system was not available for use in this study.

1.4.2 Anaesthesia and choice of anaesthetic agent.

Anaesthesia for laboratory animals should provide humane restraint and prevent the perception of painful stimuli. Surgical anaesthesia is attained when pain perception is completely suppressed. In addition, it is desirable that the animal has a decreased perception of other external stimuli such as noise, and that there is a suppression of reflex activity and loss of skeletal muscle tone. The choice of a particular method of anaesthesia depends on a variety of factors, the most important being the humane treatment of the animal and safety of the operator (Strobel & Woolman 1969, Chenoweth & Van Dyke 1969, Ben *et al* 1969).

Inhalation anaesthesia was rejected as a method for this investigation, as it is not suitable for the sustained periods required by the subsequent operative procedures (Miller 1969, Leash 1969).

Theoretical considerations led to the choice of the neurolept-analgesic combination of hypnorm and midazolam. Midazolam hydrochloride is a benzodiazepine and produces good muscle relaxation. Hypnorm is a combination of fluanisone, a neuroleptic, and fentanyl, a potent narcotic analgesic. The narcotic analgesic is included to abolish the perception of pain, and the neuroleptic, which is a tranquillizer, to suppress some of the undesirable effects of the narcotic such as vomiting or excitement. This cocktail has been widely reported as being a good, stable long-/medium- term anaesthetic with the advantage of having an analgesic action. It has also been reported as having few side effects and producing a stable respiratory and cardiovascular state (Schmitt 1969, Erhardt *et al* 1977, Flecknell *et al* 1983, Svendsen & Carter 1984).

Initial work with this anaesthetic regime produced some surprising effects; the mesenteric response to noradrenaline and electrical stimulation was less than expected (Jackson & Campbell 1980a). Further investigation led to the discovery that fluanisone (a constituent of hypnorm) is a potent α-adrenoceptor antagonist. The affinity of fluanisone for α-adrenoceptors is greater than that of the classical α-antagonist phentolamine when measured by competition with ^{3}H-WB-4101 binding (Janssen 1961, Peroutka *et al* 1976). This α-adrenoceptor activity prevented further use of this otherwise excellent anaesthetic regime in this investigation.

Pentobarbitone is probably the most widely used agent in laboratory animal anaesthesia. However, it has little analgesic action and surgical anaesthesia is attained only at doses close to those that cause respiratory failure. This can be a problem, especially when it is administered i.p., as the variability in action can lead to overdose. This agent was chosen for use in dogs, where the relative ease of i.v. administration allowed the quick induction of anaesthesia with a small dose, and maintenance with a carefully controlled infusion (Leash 1969).

The anaesthetic inactin, a thiobarbiturate, was chosen for use in the work with rats. This agent has been shown to produce relatively reliable and prolonged anaesthesia following i.p. administration to rats. The dose required to induce surgical anaesthesia is less than that causing significant respiratory depression, so fatal overdose is less likely than with pentobarbitone. The drug has been shown to produce a stable state of anaesthesia with less undesirable effects than pentobarbitone (Buelke-Sam *et al* 1978, Tucker *et al* 1982, Torlinska *et al* 1984). It has, however, been shown to cause significant alterations in kidney function (Elmer *et al* 1972, Knight *et al* 1978, Thomsen & Olesen 1981, Holstein-Rathlou *et al* 1982, Leyssac *et al* 1986).

1.4.3 Measurement of blood flow using ultrasonic Doppler probes.

The need to be able to measure flow through a blood vessel using an easy, reliable and "bloodless" method poses problems. Initially, these problems were solved by using electromagnetic flowmeters, and although these are accurate, they have a relatively large power requirement and so are unsuitable for telemetric recording. The ultrasonic Doppler flow probes are as accurate as electromagnetic versions, and are more suitable for use on very small vessels and for use with a telemetric system (Vatner *et al* 1970a, 1970b). The principle behind the Doppler flow probes is relatively simple. The Doppler effect occurs when ultrasound, transmitted by a crystal, strikes a moving particle (eg a blood cell). The particle scatters the ultrasound, causing a change in frequency which is proportional to the particle's velocity. This enables the velocity of blood passing through the cross-section of vessel beneath the probe to be calculated. Knowing this average velocity and the diameter of the vessel the flow can be computed (Arts & Roevros 1972).

1.5 An introduction to hypertension.

1.5.1 Clinical hypertension.

Systemic hypertension can be regarded as a quantitative disorder of blood pressure regulation. The simplicity of this statement is belied by the fact that the pathophysiology of hypertension is complicated. Blood

pressure is continuously variable both within a population and an individual. This makes the diagnosis of hypertension difficult. Blood pressure may be raised as a result of many conditions such as renal disease, Cushing's syndrome and primary aldosteronism. However, in about 80% of cases the cause is unknown; this is classified as essential hypertension. In its early phase (labile or borderline hypertension) it is characterised by intermittently raised cardiac output, with little change in vascular resistance. As the condition develops, the cardiac output returns to normal but the total peripheral resistance is raised.

The pathophysiological changes that occur in, or contribute to hypertension are complex. They include: changes in the number and diameter of arterioles, increase in blood viscosity, increased levels of catecholamines and angiotensin II, changes in membrane permeability, increased excitation-contraction coupling and changes in the number/ affinity of receptors (Pickering 1986). The condition is further complicated by environmental influences such as salt intake, diet, smoking and stress; a genetic predisposition also plays an important role.

Although the presence of hypertension may have little apparent effect on a patient, it is important to treat it, as it significantly lowers life expectancy. As, early in its development, there are few symptoms of hypertension, it is important that the drug regimes used to combat it are both effective and relatively free from side effects. Research into hypertension and its treatment, is necessary both to improve treatment and decrease its unwanted side effects thereby increasing patient compliance and decreasing mortality.

1.5.2 Animal models of hypertension.

The major difficulty in developing an animal model of hypertension arises from the heterogeneity of the disease. There are many animal models of hypertension, from surgically induced renal hypertension to genetically hypertensive rats. While no model can provide an exact counterpart for the human disease, genetically hypertensive rat models have several important features found in human hypertension. Despite these similarities, they provide only a "narrow" view of the wide spectrum of clinical hypertension. The overlap is however, broadened by the different genetic hypertensive rat models available.

The model of hypertension chosen for use in this work, was the spontaneously hypertensive Japanese Okamoto rat (SHR), developed in Kyoto (Okamoto & Aoki 1963). The normotensive Wistar rat, from which they were developed, were used as controls.

The time course of hypertension in these rats is similar to that in man, that is, blood pressure is initially normal and increases with age. The changes in cardiac output and peripheral resistance also follow a similar pattern to that observed in hypertensive patients (Richer *et al* 1978, 1980).

The major cardiovascular complications in primary hypertension include left ventricular hypertrophy and accelerated atherosclerosis which may lead to congestive heart failure. These same features are also present in the SHR. In addition to this, there is a great deal of evidence to suggest that there are structural abnormalities in the resistance vessels in both the SHR and in hypertensive man (Safar *et al* 1987). There is evidence that both hyperplasia and hypertrophy occur in the media of the vessel wall.

This leads to an increase in the media/lumen ratio and a decrease in wall distensibility (Seidel 1981, Mulvany 1984, Folkow *et al* 1984). The increased thickness and strength of the wall of hypertensive vessels may partly explain the increased contraction to stimuli in such vessels (Finch & Haeusler 1974, Folkow *et al* 1984). The question remains, whether the increase in both ventricular mass and vessel wall thickness, observed in hypertension, is a cause of that hypertension or merely a result of other changes. That is, do these structural changes cause the increased pressure or are they an attempt to keep wall tension constant (Laplace's law) in response to the increased pressure?

There is also widespread evidence of abnormalities in cellular ionic transport in the SHR, and these changes may play a role in the development and maintenance of hypertension. The most general manifestations of the altered ionic transport across the membrane, are an increase in passive permeability, a decrease in Ca^{++}-binding ability and a defect in the ability to accumulate Ca^{++}. These factors cause an increase in free intracellular Ca^{++}; this combined with alterations in intracellular Ca^{++}-distribution result in an increased sensitivity to Ca^{++} in the SHR (Wei *et al* 1976, Folkow *et al* 1977, Mc Carron *et al* 1981 Postnov & Orlov 1985). This, together with other alterations in cation transport across the membrane, can combine with alterations in sympathetic neurotransmission, resulting in an increase in transmitter release and postsynaptic effect (Postnov & Orlov 1985). This may be an important aspect of the cause and maintenance of hypertension in both the SHR and clinical hypertension.

Available evidence suggests that there is an increase in sympathetic nerve activity in the development and maintenance of hypertension in both the SHR and human essential hypertension (Westfall & Meldrum 1985). There are several studies suggesting that there is an increase in sympathetic

activity in the SHR; some of these indicate that there is an increase in the release of noradrenaline in the SHR (Yamamoto & Cline 1987). Other studies suggest that there is a reduction in presynaptic inhibition (Pipilli *et al* 1988) or an increase in presynaptic facilitation (Rand *et al* 1983, Cline 1985, Borkowski & Quinn 1985). While others suggest that the increase in activity is caused, at least partly, by a decrease in the re-uptake of noradrenaline in the SHR (Yamamoto & Cline 1987). The changes in sympathetic neurotransmission are complicated but very important to both the cause and maintenance of hypertension; the details that may be important in this investigation are discussed further in the next section.

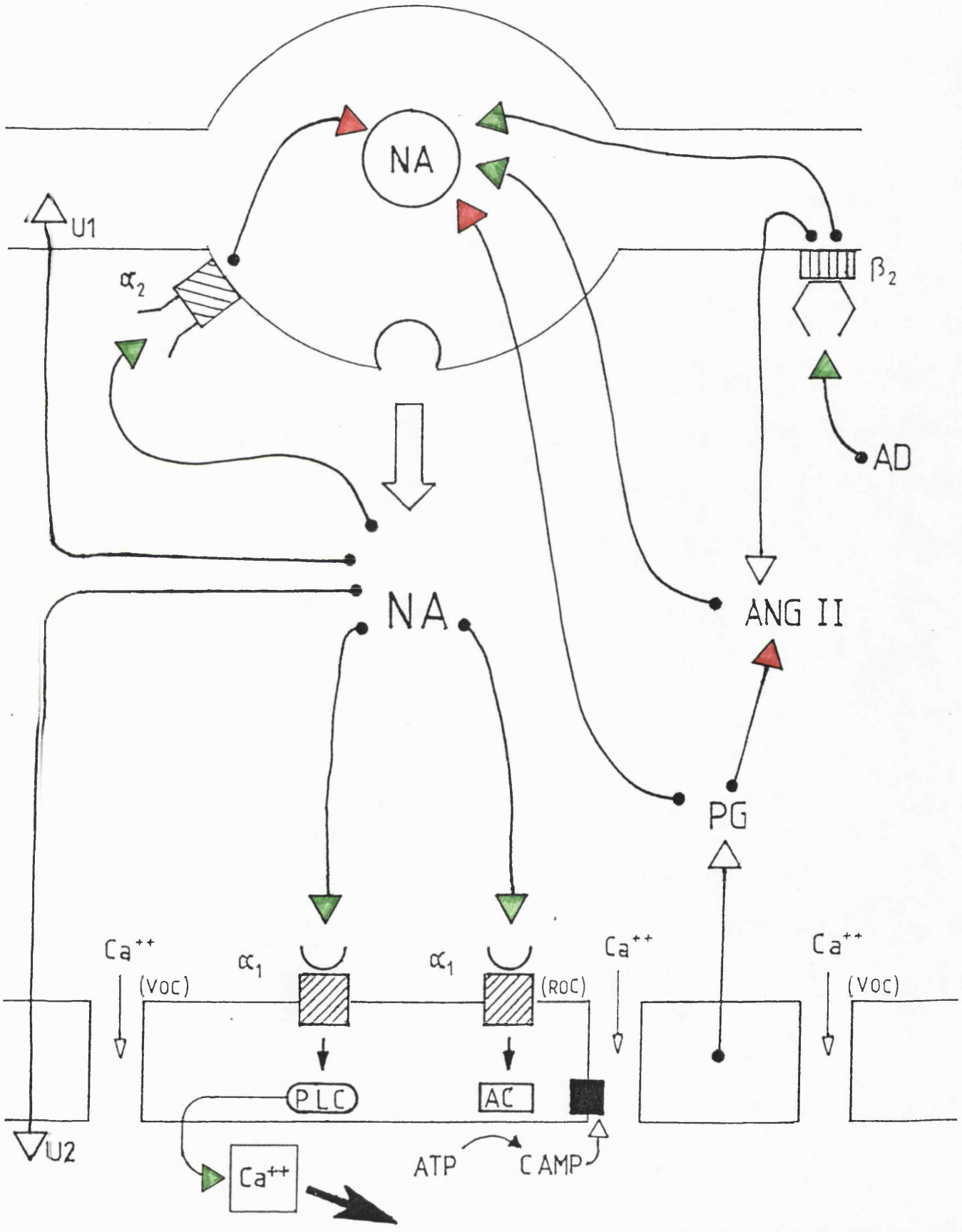
Interestingly Hallback and Folkow (1973) noted that SHR's produced a larger and more sustained sympathetic response to stress than their normotensive counterparts. They also showed that the threshold for the "defence" response to stress was lower and less likely to be inhibited by vagal restraint. As these changes were particularly apparent in the pre-hypertensive phase, they suggest that they may be a neuro-hormonal trigger for subsequent changes in structure, ionic transport and sympathetic activity, and thus be important in the initiation of hypertension. It is tempting to draw parallels between this and the involvement of stress in essential hypertension in man.

1.5.3 An introduction to noradrenergic neurotransmission, and some of its changes in hypertension.

The diagram on the following page is a simplified schematic representation of noradrenergic neurotransmission. While the detail of sympathetic neurotransmission is not all pertinent to this work, a brief description of some of the changes that have been shown to occur with hypertension will be discussed. This is especially relevant to this work as noradrenergic neurotransmission was examined using the *in situ* blood perfused mesentery model.

It is generally considered that a sympathetic action potential causes the release of noradrenaline into the synaptic cleft. This noradrenaline can then cross the cleft to interact with α_1-adrenoceptors and cause contraction of the smooth muscle in the vessel wall. The interaction with the α_1-adrenoceptors causes contraction via an increase in Ca^{++}; this may be as a result of the operation of a receptor-operated Ca^{++}-channel or the release of intracellular Ca^{++} (Bulbring & Tomita 1987). This release of noradrenaline has been shown to be increased in vessels from the SHR (Yamamoto & Cline 1987). The noradrenaline is also able to interact with presynaptic α_2-adrenoceptors which have an inhibitory effect on further transmitter release. It has been suggested that in the SHR this effect is reduced, and that noradrenaline acts almost exclusively on the postjunctional α_1-adrenoceptors (Lefevre-Borg *et al* 1988).

FIGURE 2: A schematic diagram of noradrenergic neurotransmission.



LEGEND:

NA	=	Noradrenaline
AD	=	Adrenaline
ANG II	=	Angiotensin II
PG	=	Prostaglandin
PLC	=	Phospholipid-cholesterol complex
AC	=	Adenylate cyclase
ATP	=	Adenosine triphosphate
CAMP	=	Cyclic adenosine monophosphate
ROC	=	Receptor operated calcium channel
VOC	=	Voltage operated calcium channel
U1	=	Uptake 1
U2	=	Uptake 2
α_1	=	α_1-adrenoceptor
α_2	=	α_2-adrenoceptor
β_2	=	β_2-adrenoceptor

Red arrow-head = Inhibitory effect.

Green arrow-head = Facilitatory effect.

There are also prejunctional β_2-adrenoceptors which are acted on by adrenaline to facilitate the release of noradrenaline. As there is more circulating adrenaline in the SHR this action may contribute to the development of hypertension (Rand *et al* 1983, Remie *et al* 1988). There is increasing evidence that activation of the prejunctional β_2-adrenoceptors not only has a direct effect but also causes the release of locally generated angiotensin II (Kawasaki *et al* 1984, Nakamaru *et al* 1986, Schlicker *et al* 1988).

Locally released angiotensin II has been shown to have a presynaptic effect increasing the release of noradrenaline. The exact mechanism of this action is not known, nor is the exact involvement with the presynaptic β_2-adrenoceptor (Hughs & Roth 1971, Malik & Nasjletti 1975, Jackson & Campbell 1979, 1980a, 1980b, Dzau 1984, Kaufmann & Vollmer 1985, Costa & Majewski 1988). The angiotensin II facilitation of noradrenaline release has been shown to be significantly larger in the SHR (Cline 1984, Zimmerman *et al* 1987).

There is also considerable evidence that prostaglandins are released as a result of the noradrenaline-induced activation of the vascular smooth muscle. These prostaglandins (most likely PG E_2 and possibly PG I_2) have a presynaptic inhibitory effect on noradrenaline release, either directly (Weeks 1972, Hedqvist 1973, Manku *et al* 1977, Bolton 1979, Langer 1981) or by inhibiting the angiotensin II facilitation (Jackson & Campbell 1980a, Mizuno *et al* 1988). The release of these inhibitory prostaglandins has been shown to be reduced in the SHR compared with its normotensive counterpart (Pipilli *et al* 1988).

Besides these various interactions the neuronally released noradrenaline can diffuse away from the synapse and be taken up by tissues and destroyed (uptake 2) or be taken back up into the nerve cells (uptake

1). There is evidence to suggest that there is reduced reuptake of noradrenaline in the SHR (Yamamoto & Cline 1987) allowing it to remain in the synaptic cleft longer.

In addition to these factors, other associated changes can occur in hypertension which affect noradrenergic neurotransmission. The changes in Ca^{++}-metabolism resulting in an increased Ca^{++}-sensitivity in the SHR have already been discussed. This can obviously have a great effect on the action of noradrenaline on the smooth muscle.

It should also be considered that there is increasing evidence of co-transmission in the sympathetic nervous system. There is evidence to suggest that both neuropeptide Y (NPY) and adenosine triphosphate (ATP) act as co-transmitters with noradrenaline (Campbell 1987). There is also evidence that circulating adrenaline can be taken up by the nerve and co-released with noradrenaline; and that this is more likely in the SHR (Rand *et al* 1983).

The previous section has shown that noradrenergic neurotransmission is a complicated interaction of a large number of influencing factors. Many of these factors are seen to be altered in hypertension such that they produce an increase in the effect of neurotransmission and result in greater vasoconstriction. It is possible that these changes are very important in the actual cause of essential hypertension. It is therefore imperative that such effects and the way antihypertensive drugs act on them should be investigated.

CHAPTER 2.

ATENOLOL

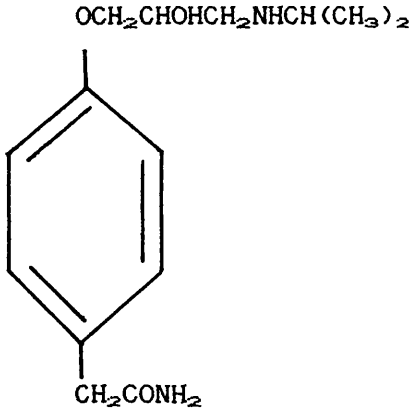
2.1 INTRODUCTION.

2.1.1 Atenolol: A brief history.

Professor Sir James Black developed the hypothesis that drugs which improved myocardial energetics in the presence of a reduced oxygen supply would improve exercise tolerance in angina pectoris and reverse or prevent catecholamine dependent arrhythmias. This led to the discovery of propranolol, a drug which has a widely accepted efficacy in angina pectoris, arrhythmias and thyrotoxicosis (Fitzgerald 1972). Clinicians were, however, concerned about the unwanted effects of propranolol, particularly the effect on respiratory β-adrenoceptors. Work on this problem led to the development of practolol, a β-adrenoceptor antagonist which was different from propranolol in four ways:- It was less potent, it was a partial agonist, it had no membrane stabilising properties and was cardioselective (Dunlop & Shanks 1968). This raised the question of whether differences in the responses to these drugs were due to adrenergic or to non-specific, non-adrenergic effects. In order to resolve this question a series of β-blockers with differing combinations of adrenergic and non-adrenergic properties was sought. One of the required pharmacological profiles, specified: a potency equivalent to that of propranolol; cardioselectivity; an absence of both partial agonism and membrane stabilising properties. The compound that most nearly met these criteria was atenolol (Tenormin, ICI 66082). The development of this cardioselective drug with high efficacy and low incidence of side effects has led to its present wide-spread clinical use.

2.1.2 Structure of atenolol.

The structure of atenolol [(4-2'-hydroxy-3'-isopropyl-aminopropoxy) phenylacetamide], has several features in common with other β-adrenoceptor antagonists, having an aromatic ring with a substituted ethanolamine side chain linked to it by an $-OCH_2$ group. The β_1-adrenoceptor selectivity is partly due to the acidic nature of the proton in the parasubstituted CH_2-$CO-NH_2$ group, although structure activity analysis shows that this is not the only determinant of cardioselectivity. The structure of atenolol is shown below.



2.1.3 Pharmacodynamic properties of atenolol.

β-adrenoceptor blocking drugs are competitive inhibitors of catecholamine binding at β-adrenoceptor sites. Studies *in vitro* using guinea pig and human atrial tissue (Harms 1976) and *in vivo* using anaesthetised cats (Carlsson 1972) show that atenolol displays a differential blockade of the chronotropic actions of isoprenaline and noradrenaline. Richardson and Witherington (1976) compared the effects of

propranolol and atenolol on isoprenaline-induced increases in hepatic blood flow in anaesthetised dogs. They found that atenolol antagonised the chronotropic actions of isoprenaline but did not affect the increases in hepatic blood flow. Atenolol is a β_1-selective blocking agent when used in low doses; it inhibits cardiac β_1-receptors but has less influence on bronchial and vascular β_2-receptors. In high doses, however, β_1-selective blockers will also block β_2-receptors (Frishman 1982).

In addition to its cardioselective effects there is some evidence of atenolol's action on other adrenoceptors. Renin release from the kidney is β-mediated and is antagonised by propranolol. Some work suggests that atenolol may affect stimulated renin release, although the effect and potency of atenolol seems to vary with species and experimental conditions. Atenolol does not significantly alter lypolysis in either human or rat adipocytes; its anti-lipolytic potency being a 50th of that of propranolol (Harms & Van der Meer 1975). Simon and Kather (1977) investigated adenylate cyclase activity in isolated human adipocytes and found that atenolol was a third as potent as propranolol in antagonising both adrenaline and isoprenaline stimulated adenylate cyclase activity.

In high concentrations certain β-blockers have a "local anaesthetic", membrane stabilising effect on the cardiac action potential. This property is exhibited equally by the two stereoisomers of a drug and is unrelated to β-blockade. Atenolol has been shown to possess no significant membrane stabilising effects; it has no significant effects on desheathed frog nerve (Fitzgerald 1977) and its (+) dextrorotatory stereoisomer has no recognised clinical value (Frishman 1979).

Certain β-adrenoceptor blockers possess partial agonist activity, these drugs cause some activation of the β-receptor in addition to their prevention of agonist access. Quantitative assessment of partial agonist

activity of atenolol made in animals whose resting sympathetic tone has been abolished by adrenalectomy and reserpine pretreatment has shown that atenolol did not have any positive intrinsic activity (Frishman 1984).

The β-adrenoceptor blockers vary in their lipid solubility, atenolol is one of the least lipid soluble β-blockers. As such it does not easily cross the blood/brain barrier and is much less concentrated in the brain than more lipid soluble drugs (Cruickshank 1980). The concentration of a drug in the brain is not necessarily important, as, in the brain fat it may not be "available" to act at central receptors. The concentration of the drug in the cerebrospinal fluid (CSF) may be more important. The concentration of atenolol in the CSF was recently measured in terms of CSF/plasma ratio and found to be low with a value of 0·25 (Taylor *et al* 1981). Lipid solubility is also important in the metabolism of atenolol; being more water soluble it is not metabolised by the liver but excreted via the kidneys. (see section 2.1.5)

2.1.4 Toxicology of atenolol.

Acute toxicological studies have shown that atenolol is well tolerated in all species and that there is no major difference between its oral and intravenous administration. The acute toxicity of atenolol following oral administration to rats, expressed as LD_{50} is 3·0gm/kg. No abnormalities were observed 14 days after acute single dose administration in surviving animals. Prolonged toxicological studies using an atenolol dose of 300mg/kg/day over six months in rats showed that there was no evidence of

any tumorigenic potential. Similar studies showed that atenolol has no mutagenic or teratogenic potential (Fitzgerald 1979).

2.1.5 Pharmacokinetics and metabolism of atenolol.

Atenolol absorption from the gut varies between species but the plasma level achieved is proportional to the dose administered. In the dog absorption is almost complete, while in the rat it is about 60%. The plasma level following oral administration is proportional to the dose administered. Peak plasma levels are observed after two hours in dogs and after two to four hours in rats. The elimination half-life (T½) is 6·5 hours in dogs and 24-35 hours in rats. Autoradiographic studies show that ^{14}C labelled atenolol is widely distributed throughout the body, with the exception of the brain and spinal cord. This is true in both rats and dogs. In the blood, atenolol is evenly distributed between red cells and the plasma (Fitzgerald 1979). In both the dog and the rat, the major route for the excretion of atenolol is in the urine. When ^{14}C labelled atenolol is given i.v., over 80% is excreted in the urine within 24 hours. The major component in the urine is unchanged ^{14}C labelled atenolol, 10-12% of the radiolabel being in the form of urinary metabolites. Less than 10% of the ^{14}C labelled material is recovered from the bile, suggesting only a minor role for biliary excretion (Reeves *et al* 1978a).

Atenolol is absorbed rapidly from the gut in man, peak plasma levels being attained 2-3 hours after oral administration. The bioavailability is between 45-55% of the dose and is not improved by its administration as a solution. Plasma proteins bind less than 30% of a dose of atenolol and there is relatively little variation between individuals given the same

oral dose. The elimination half life (T½) in man is about 6 hours, although this is increased by impaired renal function. As in animals, atenolol is widely distributed throughout the body with the exception of the brain and spinal cord. In common with rats and dogs atenolol is almost completely excreted in the urine and undergoes less than 10% biotransformation (Reeves *et al* 1978b).

2.1.6 Cardiovascular and hypotensive effects of atenolol.

The β_1-adrenoceptor specificity and potency of atenolol (discussed in section 2.1.3) provide a theoretical basis for its safe and therapeutically effective use for a variety of cardiovascular disorders.

The cardiovascular effects of atenolol depend on the dose used and the degree of autonomic activity present prior to its administration. The haemodynamic effect of i.v. administered atenolol in anaesthetised dogs is typically a fall in heart rate, and a reduction in both contractile force and aortic blood flow (Fitzgerald 1979). These effects may be explained by assuming that conduction of impulses through the atrioventricular node is modulated by autonomic activity, and that catecholamines facilitate this conduction. β-adrenoceptor blockers delay conduction by antagonising this catecholamine mediated facilitation (Frishman & Silverman 1984). Fitzgerald (1979) showed that atenolol reduced atrioventricular conductivity in anaesthetised dogs in a dose dependent manner up to a dose of 1mg/kg i.v., but had no further significant effects at higher doses. This reduction in cardiac output does not, however result in an immediate reduction in systemic blood pressure due to increased baroreceptor reflex activity and subsequent increase in peripheral resistance (Scott 1981).

The reduction in cardiac output occurs acutely after β-blockade (Gibson 1974, Fitzgerald *et al* 1978) but the fall in blood pressure is delayed (Tarazari & Dustan 1972, Antonaccio *et al* 1986). The ability of p.o. administration of single doses of various β-adrenoceptor blockers to reduce blood pressure in spontaneously hypertensive rats is controversial. Some authors found that such administration reduced blood pressure (Levy 1976, Sybertz *et al* 1982, Roba *et al* 1972, Smits *et al* 1980, Garvy & Ram 1975), while others found that it had no effect or raised blood pressure (Davy *et al* 1977, Levy 1976, Nakao *et al* 1975, Tabei *et al* 1970, Sybertz *et al* 1982). Similar contradictions in the antihypertensive effects of β-blockers exist in work using other experimental forms of hypertension. While some of these differences may be explained by variation in the methods and the pharmacology of the various β-adrenoceptor blockers used, it is obvious that the hypotensive action of atenolol (& other β-blockers) is more complicated than is suggested by the theoretical considerations explained earlier.

The hypotensive action of atenolol and other β-adrenoceptor blockers following chronic administration is less controversial. Antonaccio *et al* (1986) showed that administration of atenolol (30mg/kg p.o.) to conscious spontaneously hypertensive rats over four days produced a reduction in mean blood pressure. The onset of this reduction in blood pressure was, however, slower than the onset of β-blockade and became greater with duration of treatment. Other workers have also shown a similar delay in the hypotensive action of β-adrenoceptor blockers (Smits *et al* 1980, Scott 1981, Frishman & Silverman 1984). Esler and co-workers (1977) examined the antihypertensive effect of propranolol over a wide range of doses. They found that propranolol in plasma concentrations between 10-30ng/ml produced a modest hypotensive effect that was well correlated with a decreased cardiac output. However, when the dose was increased, a further decrease

in blood pressure was noted despite no further change in cardiac output. This and other work has suggested that the hypotensive action of atenolol (& other β-blockers) is unrelated to acute β-blockade and changes in heart rate (Antonaccio *et al* 1986). Although acute β-adrenoceptor blockade is important in the prevention of the pressor response to catecholamines with exercise and stress (Frishman & Silverman 1984). This alone does not sufficiently explain the widely accepted view that β-adrenoceptor blockers are effective antihypertensive agents in humans. The clinical efficacy of atenolol (& other β-blockers) as a antihypertensive has been well established and it is widely used for this purpose (Robertson 1983, Kaplan 1983, BÜhler *et al* 1983). There is, however, no consensus of opinion as to the mechanism(s) of atenolol's antihypertensive action.

2.1.7 Possible mechanisms of the hypotensive action of atenolol.

A number of proposed mechanisms to explain the antihypertensive action of β-adrenoceptor blockers are discussed by Frishman and Silverman (1984). They suggest that the following may play a part in the hypotensive effect of β-blockade:

1. Reduction in cardiac output.
2. Central nervous system effects.
3. Reduction in plasma volume.
4. Reduction in venomotor tone.
5. Resetting of baroreceptor levels.
6. Inhibition of renin.
7. Prejunctional effects - reducing noradrenaline release.

The reduction in cardiac output has already been discussed, and while it is undoubtedly important in preventing the pressor responses to stress and exercise it does not seem to be directly related to an antihypertensive effect. The initial reduction in cardiac output may. however, be important in the subsequent development of an antihypertensive effect, although this may not be correlated to β-adrenoceptor blockade.

Although some β-adrenoceptor blockers may exert a central hypotensive effect, atenolol is not lipid soluble and as such does not reach the brain, spinal cord or CSF in sufficient quantities to produce such an effect (Frishman 1982).

Studies have demonstrated that β-adrenoceptor blockers reduced both plasma volume and venomotor tone after both acute and chronic administration (Frishman & Silverman 1984). These studies are not yet fully substantiated, and while they are of interest they are somewhat contradictory as one would expect a reduced cardiac output to produce a reflex increase in plasma volume.

Pickering and co-workers (1972) suggest that in hypertension baroreceptors exhibit a reduced sensitivity which may be increased by β-blockade. The lack of an initial hypotensive effect with β-blockers is caused by an increase in baroreflex activity as a result of a fall in cardiac output. Subsequently the peripheral resistance falls below previous control values and blood pressure is decreased (Smits *et al* 1979) this may be as a result of a change in baroreceptor reflex. After chronic administration of propranolol Angell-James & Bobik (1978) demonstrated a reduced threshold pressure and an increased gain of individual baroreceptor fibres in hypertensive rabbits.

The relationship between the hypotensive action of β-adrenoceptor blockers and their ability to reduce plasma renin is a controversial area in hypertension research. There is no doubt that some β-blocking drugs can antagonise sympathetically-mediated renin release (Laragh 1973) but this is not the only mechanism of mediating renin release. It has been suggested that β-adrenoceptor blockers may have a hypotensive effect by decreasing plasma renin activity (Bühler *et al* 1975). Work by Antonaccio and co-workers (1986) with a number of β-adrenoceptor blocking drugs shows that while some do decrease plasma renin activity there is no correlation between this and their ability to reduce blood pressure. Furthermore they found that atenolol did not significantly alter plasma renin activity but did reduce blood pressure. This lack of correlation between β-blockade and plasma renin activity has also been noted in clinical studies (Langer *et al* 1980). It seems unlikely therefore that the hypotensive action of β-adrenoceptor blockers can be explained by simple reductions in plasma renin activity. Interestingly Kawasaki, Kline & Su (1984) suggest that a vascular renin-angiotensin system may be involved in the hypotensive effects of some β-adrenoceptor blockers.

There have been several suggestions that β-adrenoceptor blockers may act at some presynaptic site on peripheral noradrenergic neurons. Some blockers may act on presynaptic β-adrenoceptors decreasing a β-mediated facilitation of noradrenaline release (Frishman & Silverman 1984). The effects of such an interaction depends on the specificity and dose of the β-blocker used and the degree of intrinsic activity it possesses. Studies have shown that the presynaptic β-receptor resembles the β_2-subtype and stimulation causes an increase in noradrenaline release (Rand, Majewski and Tung 1983). This presynaptic β-receptor would be activated either by

circulating adrenaline, which has been shown to be raised (three fold) in hypertension (Pak 1981) or from adrenaline co-released at the synapse. There is evidence that adrenaline derived from neuronal uptake from the circulation can be co-released from sympathetic nerves (Kawasaki *et al* 1984). Atenolol, being β_1-selective and having no significant partial agonistic properties (see section 2.1.3) would not be likely to interact with presynaptic β-receptors. Additionally the time course of the antihypertensive action of β-blockers does not seem to be correlated with β-blockade.

Some workers have suggested that β-blockade can affect the turnover of noradrenaline (Alexandre & Chevillard 1980) or inhibit its uptake (Street & Walsh 1984). These effects seem to depend on lipid solubility and membrane stabilising effects and are therefore not likely to be the main mode of atenolol's hypotensive action.

Scott (1981) discovered that atenolol reduced sympathetic efferent discharge, and attenuated the reflex responses of the sympathetic nerves to changes in blood flow in her work with anaesthetised cats. This suggested that atenolol acted on sympathetic nerves outside the CNS possibly either as a result of changes in the baroreceptor reflex arc or some influence at transmission at sympathetic ganglia.

Jackson and Campbell (1979, 1980a 1980b) have shown that angiotensin II in sub-pressor doses inhibited neuronal noradrenaline uptake and facilitated its release (Campbell & Jackson 1979). Some workers have suggested that β-adrenoceptor blockers may act by increasing the vascular production of prostaglandins, which in turn act to inhibit the angiotensin II potentiation of adrenergic transmission (Jackson & Campbell 1980a, 1981, Daniell *et al* 1988). This is an attractive proposition as the effect appears to be independent of β-blockade and has been shown in a number of

species and preparations. This theory is supported by the clinical observations that treatment with drugs such as indomethacin which inhibit the production of prostaglandins can attenuate the effect of β-blockade on blood pressure (Durao *et al* 1977a, 1977b, Watkins *et al* 1980, Salvetti *et al* 1984).

The effect of β-adrenoceptor blockers on the sympathetic nervous system is complex. There is a great deal of evidence suggesting that β-blockers interact with the system after chronic administration, and that the time course of this interaction may be important in their hypotensive effects. The exact interactions and the significance of acute β-blockade has yet to be determined.

In addition to the possible hypotensive mechanisms of atenolol previously discussed, there is also an argument as to its involvement in changing the structural abnormalities involved in hypertension. Hypertensive animals (& man) have long been recognised as having ventricular and atrial hypertrophy &/or hyperplasia. There is argument as to whether this is as a direct result of the hypertension (Laplace's Law) or if it contributes to the cause and development of the hypertension. There is also some controversy as to whether β-blockade can prevent &/or reduce the structural abnormalities by reducing the cardiac output and so removing the stimulus for its development (Mulvany 1984, Folkow *et al* 1984, Richer *et al* 1978). The importance of this effect depends on whether the structural abnormalities cause, or arise from, hypertension.

The therapeutic efficacy and safety of atenolol has been well established in patients with a variety of cardiovascular disorders. It is recognised as being effective in reducing the blood pressure of many patients with systemic hypertension and is widely used clinically for this purpose. There is, however, no consensus as to the mechanism(s) of action whereby atenolol has a hypotensive effect. While there is agreement on the reduction in cardiac output obtained with acute β-blockade, this does not adequately explain the hypotensive effect observed chronically. The mechanism(s) of action involved in this long-term reduction in blood pressure was investigated in this work.

2.2 METHODS.

2.2.1 Investigation of blood pressure in the conscious rat.

The first study of blood pressure was undertaken with normotensive male Wistar rats (University of Bath strain) weighing approximately 200g. Systolic blood pressure was measured using a non-invasive "tail-cuff" procedure.

Animals were maintained in a warming chamber at 39°C for 10 minutes to dilate the caudal arteries. A pneumatic cuff was placed around the base of the tail and a sensitive pressure transducer was placed immediately distal to this. An electrosphygmomanometer (type 8002, W+W Ltd) was used to inflate and deflate the pressure cuff at a constant rate and to record the tail pulse measured by the pressure sensor. This was used to record three measurements of blood pressure, the mean of which was noted. Animals were minimally restrained throughout the procedure and extraneous activity in the laboratory was minimised to reduce stress.

Blood pressure was measured before and two hours after the daily dose of either atenolol or polyethylene glycol (PEG). During the first week of dosing all animals were treated with 5% PEG alone; this allowed them to become accustomed to the procedures involved. In the subsequent three weeks the treated group received 50mg/kg atenolol in a 5% PEG vehicle, while the control group received the PEG vehicle alone. Drugs were orally administered (1ml/100g) daily; blood pressure was not, however, measured over weekend periods. Care was taken to ensure the correct fitting and placement of both the pneumatic cuff and the pressure sensor. The

temperature and duration of pre-warming was consistent throughout the experiment. The warming chamber was thermostatically maintained at 39°C and water was available *ad libitum* enabling animals to drink and also providing a reasonable level of humidity.

This protocol was repeated using spontaneously hypertensive Japanese Okamoto rats (University of Bath strain) weighing approximately 200g.

2.2.2 Investigation of heart rate in the conscious rat.

Heart rate was calculated by counting the pulse pressure waves recorded by the pressure sensor on the proximal portion of the tail during the measurement of blood pressure described previously. These were counted over a 10 second period and multiplied by 6 to give heart rate in beats per minute. In each instance pulse pressure waves recorded before the tail cuff was sufficiently inflated to impede blood flow were used in the calculation. This was carried out on five traces for each animal and the mean value recorded.

This protocol was repeated using both normotensive Wistar rats and spontaneously hypertensive Japanese Okamoto rats (university of Bath strains) weighing approximately 200g.

2.2.3 Assessment of β-adrenoceptor blockade.

β-adrenoceptor blockade was assessed using an anaesthetised rat preparation to establish a dose response curve to bolus i.v. injections of isoprenaline.

Male Wistar rats (University of Bath strain), weighing 275-300g were anaesthetised with sodium thiobutobarbitone (Inactin; BYK) at a dose of 120mg/kg administered i.p.. The trachea was exposed and cannulated with polythene tubing (200/300/060, Portex Ltd) to facilitate ventilation.

The left carotid artery was catheterised with a polythene tube (ref: 200/300/030, Portex Ltd) which was connected to a pressure transducer (Type PD CR 75, Druck), the whole unit being filled with heparinised saline (100 units/ml 0·9% w/v NaCl). The transducer was coupled to a blood pressure pre-amplifier (type 7179, NarcoBio-Systems), the output of which was fed to a heart rate coupler (type 7302, NarcoBio-Systems) which derived heart rate from the blood pressure pulse. The pressure and heart rate output was recorded on a Physiograph pen recorder (Mk III-S, Narco).

The left jugular vein was catheterised with a saline-filled polythene tube (ref: 200/300/020, Portex Ltd) to allow intravenous injection of isoprenaline.

Body temperature was maintained at 37°C using a thermostatically controlled heated table (type 50-1247, SRI Ltd). Following a 5 minute stabilisation period bolus i.v. doses of isoprenaline (25-200ng in 0·9% w/v NaCl) were administered via the venous cannula. Responses were measured in terms of increase in heart rate, which was allowed to return to control levels between doses of isoprenaline.

This protocol was used to assess β-adrenoceptor blockade following 7 and 21 days pretreatment with either atenolol (50mg/kg p.o. in 5% PEG, 1ml/100g) or 5% PEG (1ml/100g). The procedure was followed 24 hours after the last dose of either atenolol or PEG.

2.2.4 The *in-situ* blood perfused rat mesentery.

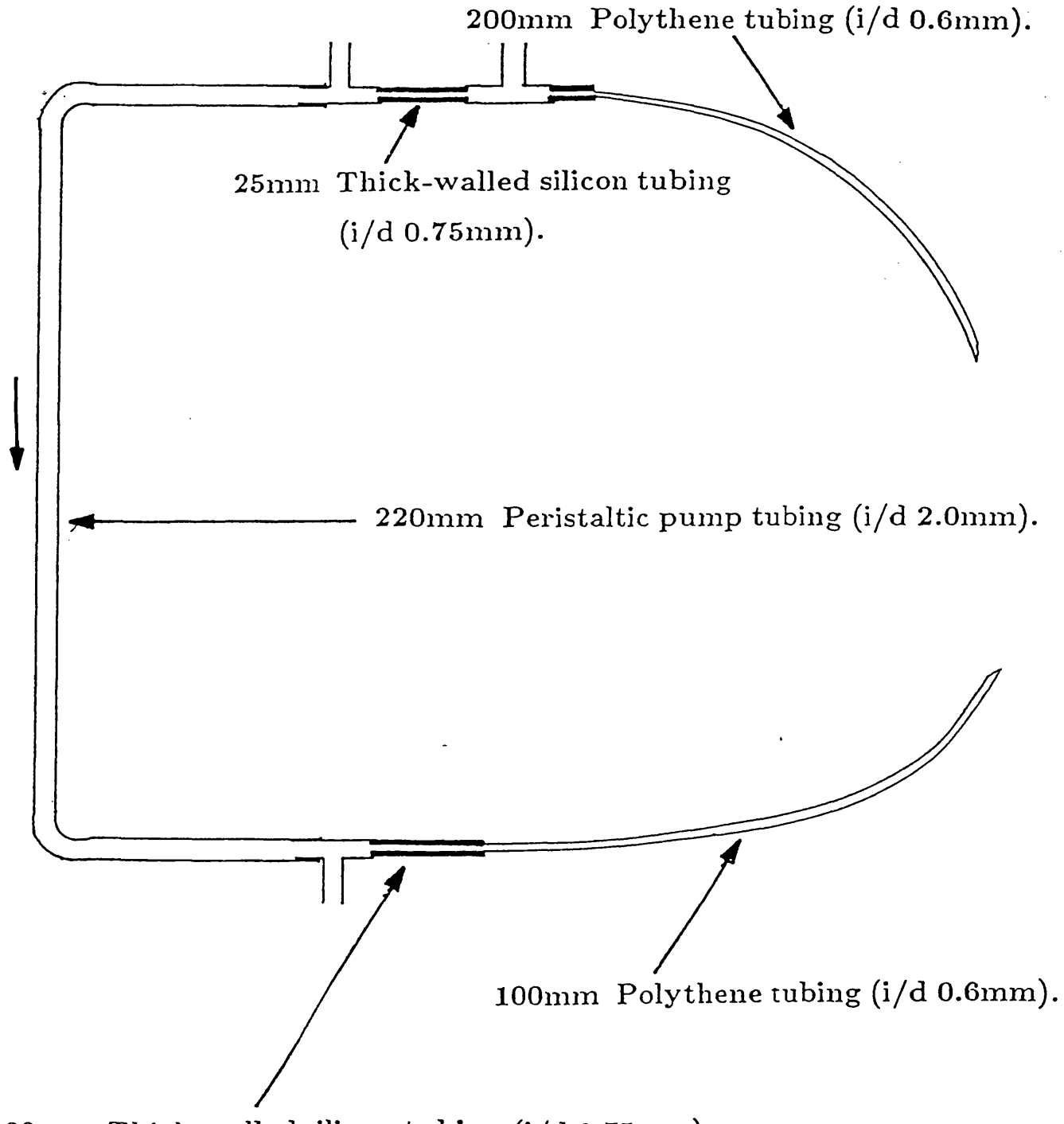
The effect of chronic β-adrenoceptor blockade on adrenergic neurotransmission was investigated using the *in-situ* blood perfused mesentery method of Jackson and Campbell (1980a).

Animals were anaesthetised with a single bolus i.p. injection of sodium thiobutobarbitone (120mg/kg, Inactin, BYK). The trachea was exposed and cannulated with polythene tubing (200/300/060, Portex Ltd). The mesentery was exposed by a midline incision, the intestines were exteriorised and wrapped in saline moistened gauze. The abdominal aorta and superior mesenteric artery were carefully exposed and cleared ready for ligation. The wound was covered with saline moistened gauze and the preparation was left for 10 minutes to allow haemostasis.

Heparin (1500 units) was administered via the tail vein. The lower abdominal aorta was then catheterised distal to the left renal artery permitting connection to an extracorporeal circuit. The circuit was allowed to fill with blood and the free end used to cannulate the superior mesenteric artery. (details of the extracorporeal circuit are shown diagrammatically in figure 3) A peristaltic pump (type P3, Pharmacia) was used to perfuse the mesentery at a constant 2ml/min and physiological saline (0·9% w/v NaCl) was infused into the circuit at 0·1ml/min (Infusion

FIGURE 3

Diagram showing the details of the extracorporeal circuit used in the *in-situ* blood perfused mesentery method.



pump 5200, SRI Ltd). During the cannulation procedure the mesenteric vascular bed was subjected to a brief ischaemic period of less than 2 minutes. Bipolar platinum electrodes were placed around the superior mesenteric artery about 3mm distal to the cannulation point. Finally the abdomen was covered in saline-moistened gauze and the preparation was allowed to stabilise. Throughout the whole procedure the animal's body temperature was maintained at 37°C using a thermostatically controlled heated table (type 50-1247 SRI Ltd).

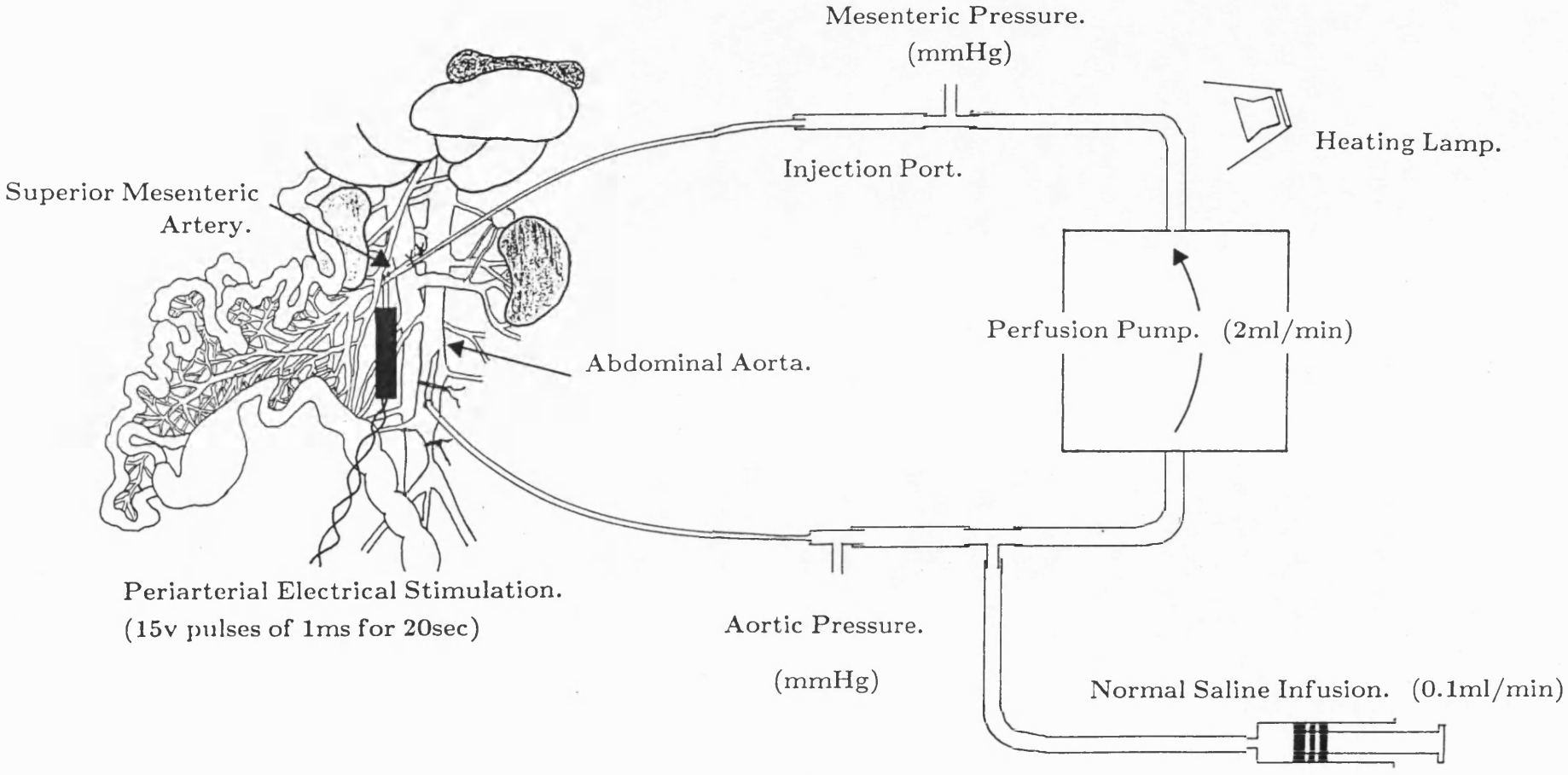
Pressure was recorded in the circuit before the perfusion pump, to give a measure of aortic blood pressure, and after the pump to give a measure of mesenteric pressure. Pressure transducers (type PD CR 75, Druck) coupled to pressure pre-amplifiers (type 7179, Narco) recorded pressure changes on a pen recorder (Physiograph mk III-S, Narco). Changes in pressure were indicative of changes in mesenteric vascular resistance in this constant flow system.

Figure 4 shows a diagrammatic representation of the *in-situ* blood perfused mesentery method.

This protocol was used to investigate mesenteric response to exogenous noradrenaline (20-2000ng in 0·9% w/v NaCl) and periarterial electrical stimulation (15v rectangular pulses of 1ms duration for 20s, 2-35Hz). This was undertaken 24 hours after the last dose of either atenolol (50mg/kg in 5% PEG, 1ml/100g) or 5% PEG (1ml/100g) after both 7 and 21 days pretreatment. The work was carried out with both normotensive male Wistar and spontaneously hypertensive Japanese Okamoto rats (University of Bath strain, 300-330g).

FIGURE 4

A diagrammatic representation of the *in-situ* blood perfused mesentery method.


Mesenteric Pressure.
(mmHg)
Heating Lamp.
Injection Port.
Superior Mesenteric
Artery.
Perfusion Pump. (2ml/min)
Abdominal Aorta.
Periarterial Electrical Stimulation.
(15v pulses of 1ms for 20sec)
Aortic Pressure.
(mmHg)
Normal Saline Infusion. (0.1ml/min)

2.2.4(i) Adaptation of the *in-situ* blood perfused mesentery method to allow a blood-Krebs comparison.

The method described in section 2.2.4 was modified to allow a comparison between blood- and Krebs-perfused *in-situ* mesentery methods. The extracorporeal circuit shown in figure 4 was modified such that the saline infusion line could be used for the infusion of Krebs. This adaptation is illustrated in figure 5.

The protocol was carried out as previously described (section 2.2.4) using normotensive male Wistar rats (Alderly park strain, 300g). Responses were obtained to exogenous calcium chloride (10-100mg) or periarterial electrical stimulation during blood perfusion. The supply of blood from the abdominal aorta was then stopped by clamping the silicon tubing proximal to the saline infusion line. Krebs at 37°C gassed with 95% O_2 and 5% CO_2 was supplied via the saline infusion line. The hepatic portal vein was ligatured and cut to allow the drainage of the Krebs from the mesenteric vascular bed. The system was allowed to stabilise for 10 minutes and the responses to exogenous calcium chloride (10-100mg) or periarterial electrical stimulation were re-examined.

Appropriate time controls (n=4) revealed that there was no significant difference in blood perfused responses to calcium chloride or periarterial electrical stimulation over the 30-45 minutes required to obtain the results during perfusion with Krebs. Further blood and Krebs comparisons were made in the same animals; a group of three animals was used to compare response to calcium chloride and a group of eight animals used to compare response to electrical stimulation.

FIGURE 5

A diagrammatic representation of the *in-situ* blood perfused mesentery method, showing its adaptation to allow blood-Krebs comparison.

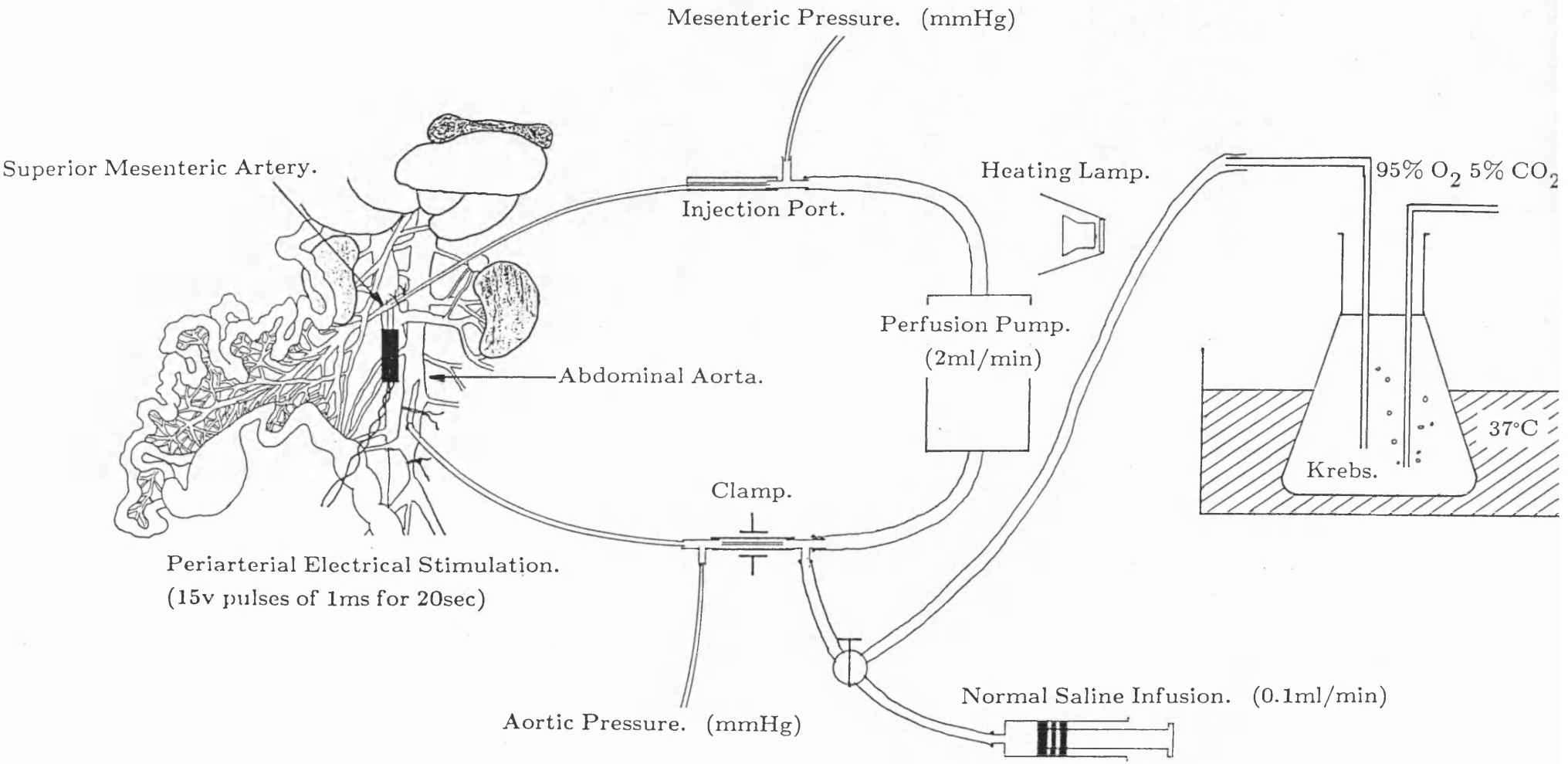

Mesenteric Pressure. (mmHg)
Superior Mesenteric Artery.
Injection Port.
Heating Lamp.
95% O_2 5% CO_2
Perfusion Pump.
(2ml/min)
Abdominal Aorta.
37°C
Krebs.
Clamp.
Periarterial Electrical Stimulation.
(15v pulses of 1ms for 20sec)
Normal Saline Infusion. (0.1ml/min)
Aortic Pressure. (mmHg)

Table 1 shows that there is no significant difference in response to exogenous calcium chloride in blood- and Krebs-perfused systems.

Table 1: Response to exogenous calcium chloride. (mmHg)

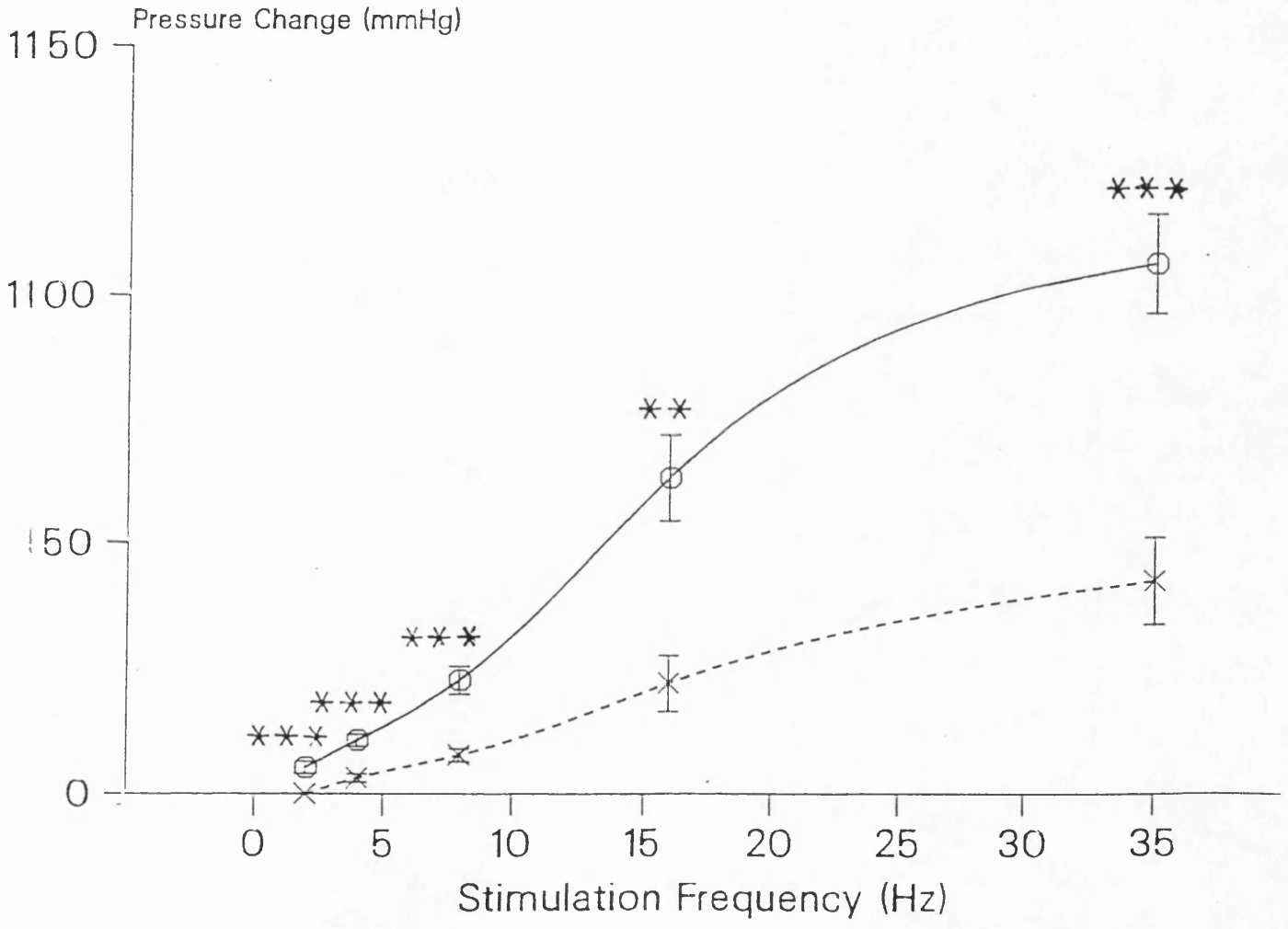
	BLOOD PERFUSED				KREBS PERFUSED			
[$CaCl_2$]				mean±SE				mean±SE
10mg	5·0	10·0	10·0	8·3±1·4	5·0	20·0	0·0	8·3±4·9
20mg	10·0	20·0	5·0	11·7±3·6	10·0	30·0	2·0	14·0±6·8
40mg	15·0	20·0	15·0	16·7±1·4	15·0	20·0	7·0	14·0±3·1
100mg	10·0	----	15·0	12·5±1·8	15·0	35·0	10·0	20·0±6·2

Figure 6 is a graphical representation of the results of the comparison in response to electrical stimulation. This indicates there is a highly significant ($p<0·001$) reduction in response in the Krebs perfused system. The decrease in base-line perfusion pressure (approx. 70mmHg) experienced during Krebs perfusion may partly explain this result, although it did not appear to influence the response to calcium chloride.

The results of this work supported the decision to use the blood perfused system for all further work.

FIGURE 6

Graph showing the mesenteric response to periarterial electrical stimulation in normotensive male Wistar rat during perfusion with blood or Krebs solution.



o——o Blood perfused mesentery (n=8)

X----X Krebs perfused mesentery (n=8)

Difference from corresponding value in Krebs perfused preparation :

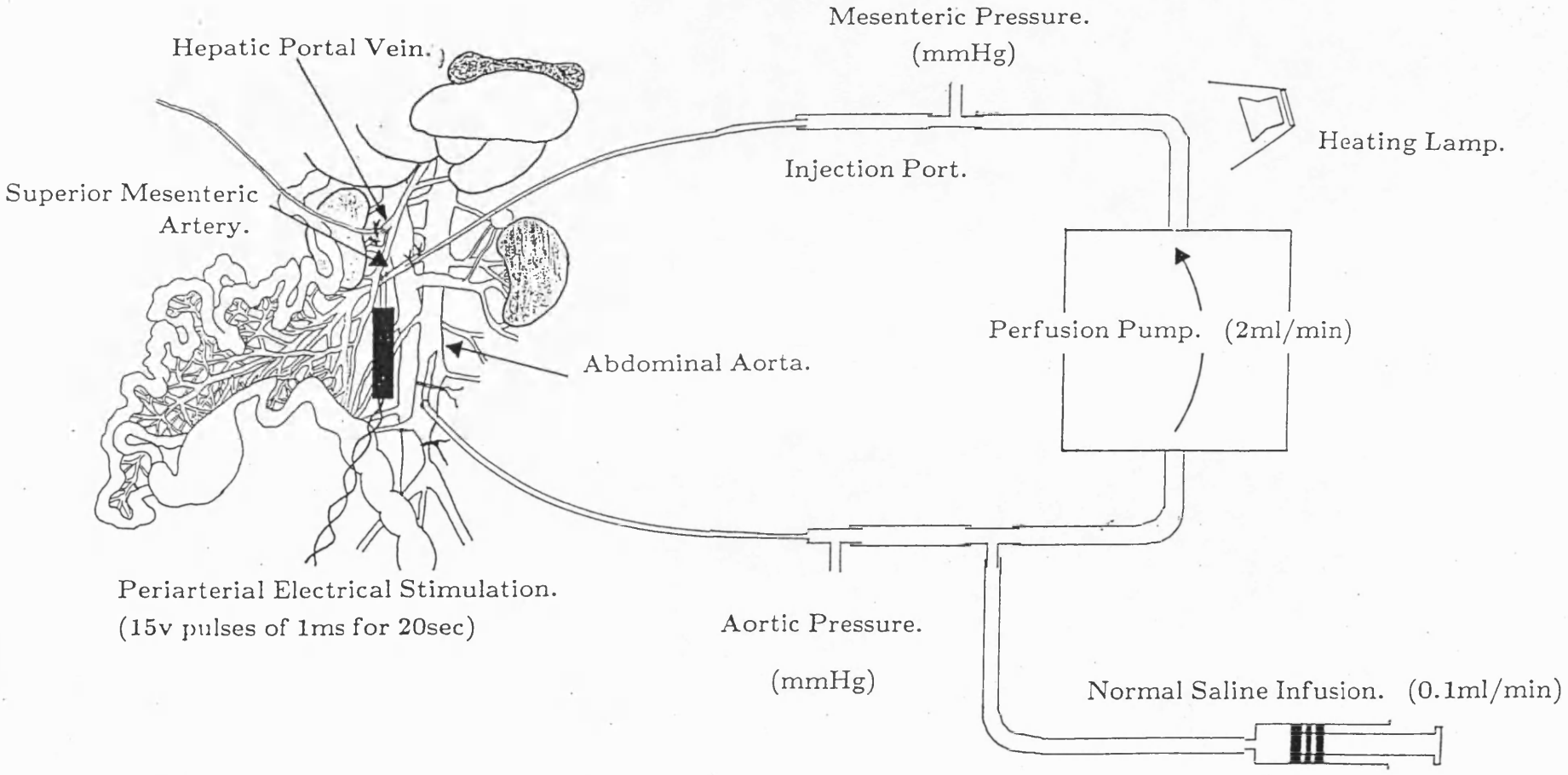
* p<0·05 ** p<0·01 *** p<0·001

2.2.5 Investigation of stimulus-induced ^{3}H-noradrenaline overflow.

The *in-situ* blood perfused mesentery method was also further modified in some later experiments to allow serial blood sampling from the hepatic portal vein. A fine polythene tube (200/300/020, Portex) was used to catheterise a side-branch of the hepatic portal vein such that the end was positioned in the main flow of the mesenteric venous drainage. This modified technique is illustrated in figure 7.

FIGURE 7

A diagrammatic representation of the *in-situ* blood perfused mesentery method, showing its adaptation to allow the investigation of ^{3}H-noradrenaline overflow.


Hepatic Portal Vein.
Mesenteric Pressure.
(mmHg)
Heating Lamp.
Injection Port.
Superior Mesenteric
Artery.
Perfusion Pump. (2ml/min)
Abdominal Aorta.
Periarterial Electrical Stimulation.
(15v pulses of 1ms for 20sec)
Aortic Pressure.
(mmHg)
Normal Saline Infusion. (0.1ml/min)

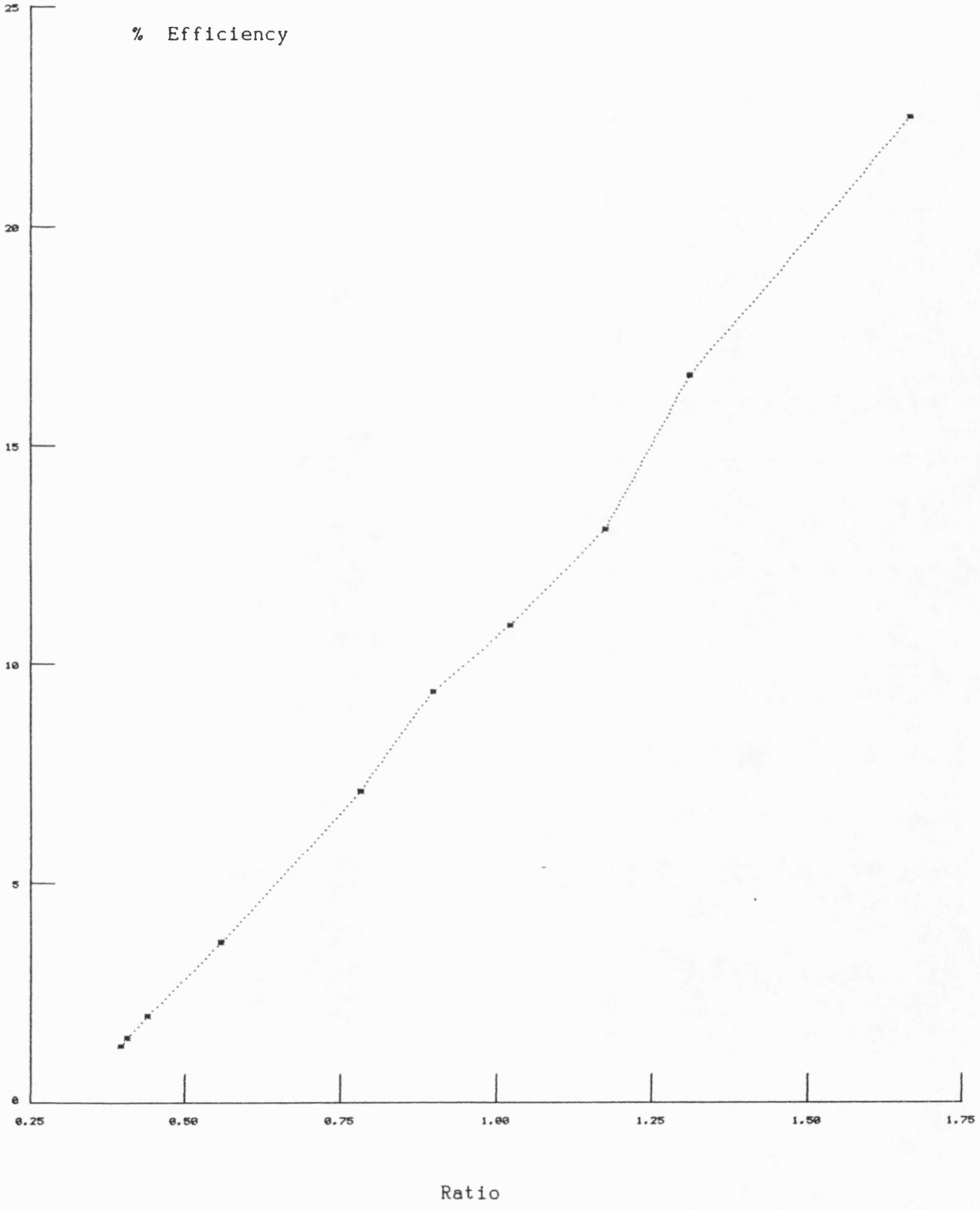
2.2.5(i) Construction of a quench curve for tritium.

Previous studies had shown that whole blood was likely to produce the greatest amount of quenching because of the strong colour effect exhibited at the red end of the spectrum. To compensate for this, a standard quench curve was calculated and stored to be used in the automatic correction of subsequent readings. Calculation and storage of the quench curve was carried out automatically in the Rackbeta scintillation counter using the following method:-

Ten vials were prepared, a standard amount of tritium (1210-120, LKB Wallac) was added to each vial. The multipurpose liquid scintillation cocktail, Optiphase 'Safe', which is suitable for use with both aqueous and non-aqueous samples (LKB Scintillation Products), was added to each vial (10ml). The first vial was then capped without the addition of any quenching agent. Increasing amounts of untreated whole blood was then added to the other nine tubes. Each tube contains the standard tritium, 10mls of scintillation cocktail and the following volumes of whole blood: 0µl, 5µl, 10µl, 15µl, 20µl, 30µl, 50µl, 75µl, 100µl and 150µl. Following the addition of the blood, the vials were capped and the samples loaded into the scintillation counter for quench calibration. The counts in each vial were measured and the quench calibration was calculated and stored in the counter's memory.

The tritium quench curve is shown in figure 8 on the following page.

FIGURE 8: Tritium quench curve.



2.2.5(ii) Sample preparation.

Before each experiment clean vials were prepared each containing 2ml of Optiphase 'Safe'. As each 20µl blood sample was taken it was placed into a vial which was capped and mixed well. At the end of each experiment all the vials were whirli-mixed for 30 seconds, placed into a rack and loaded into the liquid scintillation counter (Rackbeta 1215, LKB Wallac) for counting. Each sample was counted for 5 minutes against the internal control using the quench calibration previously calculated.

2.2.5(iii) Investigation of the time course of stimulus induced tritiated noradrenaline overflow.

Throughout the investigation of tritiated noradrenaline overflow the radioisotope L-(7,8) noradrenaline hydrochloride (Amersham International PLC) was used. This isotope was supplied with an activity of 9·25 MBq at a concentration of 1·0 mCi/ml.

The *in-situ* blood-perfused mesentery was prepared as previously described (2.2.4) with the additional cannulation of the side branch of the hepatic portal vein. The venous cannula was filled with heparinised saline (100 u/ml 0·9% w/v NaCl) and connected to a 100µl microsyringe (Hamilton). The mesentery was loaded with 2·5µci ^{3}H-noradrenaline in 5ml of saline in a 25 minute infusion. Desmethylimipramine (DMI, 2·5mg/kg in 0·9% w/v NaCl)

was added through the infusion line and the tritiated noradrenaline infusion replaced with a saline (0·9% w/v NaCl) infusion (0·1ml/min).

After 5 minutes a 20µl blood sample was taken from the hepatic portal vein and placed in a vial. Three minutes later the superior mesenteric artery was stimulated at 16Hz (15v rectangular pulses of 1ms duration for 20s) via the periarterial platinum electrodes. Blood samples (20µl) were then taken 30 seconds, 1 minute, 2 minutes, 3 minutes, 6 minutes and 10 minutes after the start of stimulation. Three minutes after the last blood sample the periarterial electrical stimulation was repeated and blood samples were taken 30 seconds, 1 and 2 minutes after stimulation. The time course of the sampling procedure is summarised in the following table:-

TIME	ACTION
-33 min	Start ^{3}H-noradrenaline infusion.
-8 min	Stop ^{3}H-noradrenaline infusion
	Add DMI (2·5mg/kg)
	Start saline infusion.
-3 min	Sample 1.
0	Stimulation 16Hz - ON.
+20 s	Stimulation - OFF.
+30 s	Sample 2.
+1 min	Sample 3.
+2 min	Sample 4.
+3 min	Sample 5.
+6 min	Sample 6.
+10 min	Sample 7.
+13 min	Stimulation 16Hz - ON.
+13m 20s	Stimulation - OFF.
+13m 30s	Sample 8.
+14 min	Sample 9.
+15 min	Sample 10.

These samples were then prepared and measured as previously explained in section (ii).

This work was undertaken using a group of 4 normotensive male Wistar rats (University of Bath strain) weighing approximately 300g. The following table shows the mean of the results obtained:-

SAMPLE	MEAN DPM	SAMPLE	MEAN DPM
sample 1	2277·6	sample 7	2457·3
sample 2	**4047·5**	sample 8	**3004·8**
sample 3	2926·5	sample 9	1944·7
sample 4	2564·8	sample 10	1889·1
sample 5	2430·0		
sample 6	2924·3		

These results show that the highest level of radiolabel was observed in samples collected 30 seconds after electrical stimulation. The release of tritiated label was apparent after a second stimulation but at a reduced level. These results led to the conclusion that in all further work blood samples should be taken 30 seconds, 1, 2 and 3 minutes after stimulation in order to observe the stimulus induced overflow.

2.2.5(iv) Investigation of the effect of chronic atenolol pretreatment on stimulus-induced ^{3}H-noradrenaline overflow.

The *in-situ* blood perfused mesentery, with the additional cannulation, was set up as described previously. The mesentery was loaded with 2·5µci ^{3}H-noradrenaline as described in section (iii); DMI (2·5mg/kg) was added and the tritium infusion replaced with a saline (0·9% w/v NaCl) infusion.

After 5 minutes a 20µl blood sample was removed from the hepatic portal catheter and placed in a vial. After a further 3 minutes the superior mesenteric artery was stimulated at a frequency of 16Hz (15v rectangular pulses of 1ms duration for 20s). Further blood samples were

taken 30 seconds, 1 and 3 minutes after stimulation. The time course of the sampling procedure is shown in the table below.

TIME	ACTION
-33 min	Start ^{3}H-noradrenaline infusion.
-8 min	Stop ^{3}H-noradrenaline infusion
	Add DMI (2·5mg/kg)
	Start saline infusion.
-3 min	Sample 1.
0	Stimulation 16Hz - ON.
+20 s	Stimulation - OFF.
+30 s	Sample 2.
+1 min	Sample 3.
+2 min	Sample 4.
+3 min	Sample 5.

The samples were prepared and measured as previously described. Stimulus-induced radio-label release was shown by the difference in DPM measured before stimulation (sample 1) and the maximum levels recorded after stimulation (sample 2). This difference was compared between animals pretreated for 21 days with either atenolol (50mg/kg in 5% PEG) or 5% PEG (1ml/100g). This work was undertaken in male normotensive Wistar rats (University of Bath strain, 300g); 24 hours after pretreatment.

2.2.6 Investigation of the effect of chronic atenolol pretreatment on mesenteric vascular responses in normotensive Beagles.

Normotensive male Beagles (Alderly park strain, 17-20kg) were anaesthetised with sodium pentobarbitone (30mg/kg, i.v.). The right femoral vein was catheterised (500/100/260, ID=1·5mm, OD=2·5mm; Portex Ltd) and used to administer an infusion of sodium pentobarbitone (1mg/min in 0·9% w/v NaCl) to sustain anaesthesia. An endotracheal tube was inserted and the animals ventilated with room air. The right femoral artery was exposed, catheterised (Portex polythene tube ref 500/100/260) and used to measure systemic blood pressure. This was achieved by attaching the cannula to a pressure transducer (PD CR 75, Druck) coupled to a Devices pressure pre-amplifier. Heart rate was derived from a lead II electrocardiogram (ECG) using a cardiotachometer (Devices). The left femoral artery was catheterised (Portex Ltd, ref 500/100/260) to facilitate the intermittent sampling of arterial blood. This was used for the determination of blood pH, pO_2 and pCO_2 which were adjusted to normal values as required. Blood gasses were measured using a blood gas analyser (Corning 165) using freshly drawn arterial blood sealed against the atmosphere.

A laparotomy was performed and the superior mesenteric artery was located and carefully cleared to allow the location of a flow probe. The flow probe was located as near the base of the vessel at the junction with the abdominal aorta as possible. This section of vessel was well cleared of connective tissue to allow the correct fitting of the probe. The diameter of the probe used was selected so as to provide a 'snug' fit without occluding the vessel. Bipolar platinum electrodes were placed around the vessel distal to the probe. The electrodes were located on a

section of vessel that was minimally cleared to allow access without disturbing the periarterial nerves. A suitable side-branch of the superior mesenteric artery was located and cleared to permit cannulation. This was then catheterised with polythene tubing (200/300/030, Portex Ltd) to allow the administration of drugs and the measurement of mesenteric blood pressure. Mesenteric blood pressure was recorded using a pressure transducer (PD CR 75 Druck) coupled to a pre-amplifier (Devices). Figure 9 (page 58) shows the location of the probe, electrodes and the cannula on the superior mesenteric artery. The abdominal wound was then closed with clamps and covered with saline moistened gauze. Throughout the whole experimental procedure the animal's body temperature was maintained at 37°C using a thermostatically controlled heating blanket. Temperature was monitored and the heating blanket controlled via a rectal temperature probe. Following a 30 minute stabilisation period mesenteric vasoconstrictor responses to exogenous noradrenaline (1-400ng in 0·9% w/v NaCl) and periarterial electrical stimulation (15v rectangular pulses of 1ms duration for 1 min, 2-35Hz) were obtained. Systemic blood pressure, heart rate, ECG, mesenteric blood pressure and mesenteric flow were displayed on a Devices (M19) pen recorder. Mesenteric resistance was calculated from this data using the following formula:

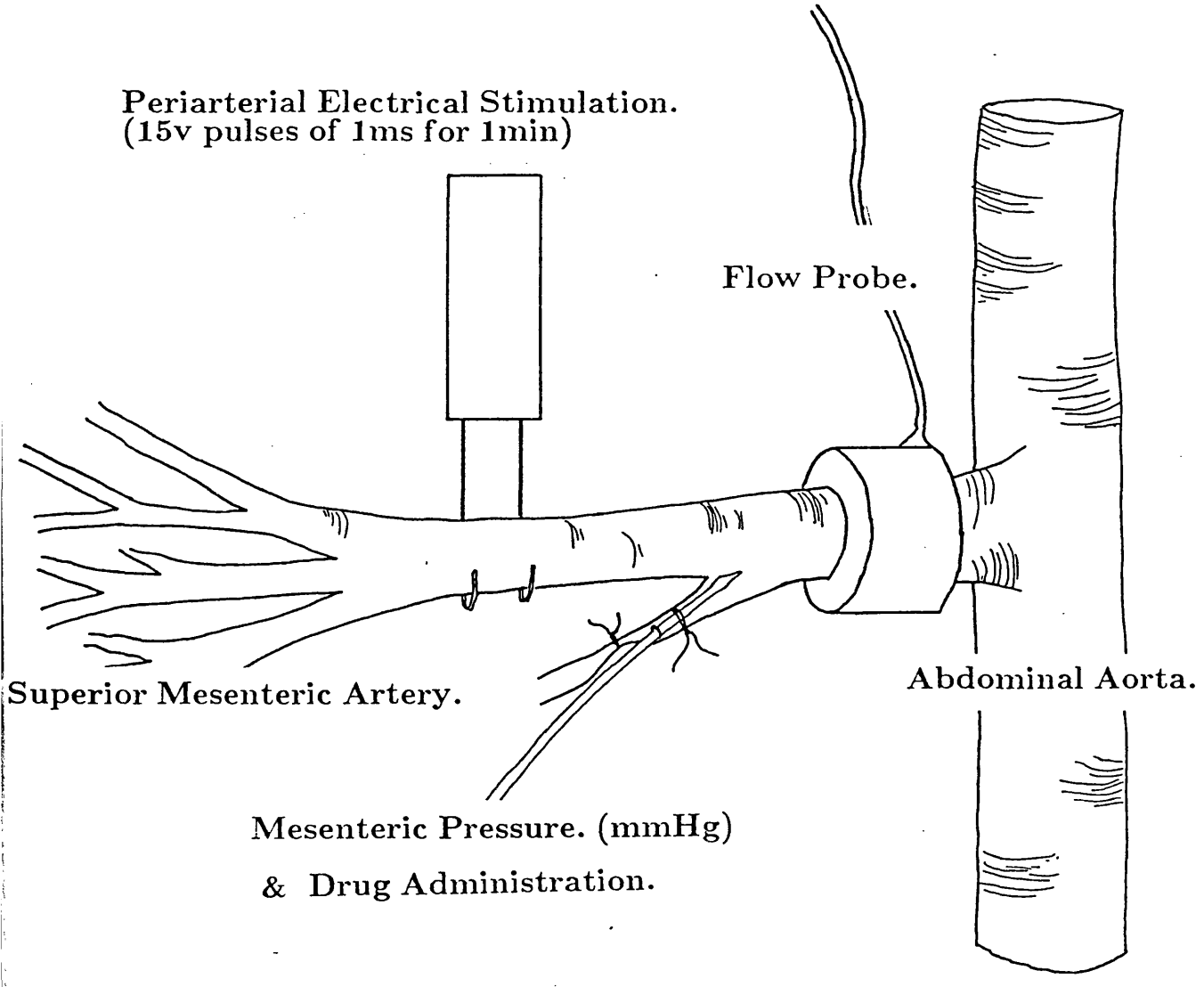
R_M (dyns/sec/cm^5) = [mean BP_M (mmHg) × 1330] ÷ F_M (mls/sec)

Where : R_M = mesenteric resistance, BP_M = mesenteric blood pressure and F_M = mesenteric flow.

This investigation was undertaken with dogs pretreated for 21 days with atenolol at a dose of 7·5mg/kg. The atenolol was administered daily as a solid in a gelatin capsule. The surgical procedure was carried out 24 hours after the last dose of atenolol and untreated animals were used as controls.

FIGURE 9

Diagram showing the placement of probes on the mesenteric artery of the normotensive male Beagle.



2.2.7 General considerations.

2.2.7(i) Animal husbandry.

Rats at the University of Bath were all housed under similar conditions. A 12 hour light and dark cycle was used and both housing and experimental rooms were kept at 20-22°C with a relative humidity of 40-60%. Animals were provided with 'Labsure' CRM diet and tap water *ad libitum*. Rats were housed in groups, so that the stress either of overcrowding or of solitude was avoided (groups of between 4 and 8).

Stock rats at Alderly park are housed under similar conditions with a 12 hour light and dark cycle, a temperature of 21 ±2°C and a relative humidity of 55±10%. Animals were provided with PCD 5ths SQC from 'Special Diet Services' and tap water *ad libitum*, and were housed in groups.

Dogs at Alderly park are kennelled with a 12 hour light and dark cycle at a temperature of 19 ±2°C, and a relative humidity of 55 ±10%. Dogs were supplied with water *ad libitum* and fed with 400g Lab A diet (Special Diet Services) per day.

When animals had to be moved from a breeding or holding colony they were kept for a week before they were used in any experimental procedures to allow them to become accustomed to the new quarters.

Surgical procedures undertaken in both rats and dogs were carried out under a general anaesthetic. The depth of anaesthesia was assessed by observing respiration, systemic blood pressure, heart rate and muscle tone.

The "pinch" reflex, the "blink" reflex and the "leg withdraw" reflex were also used to gauge the depth of anaesthesia.

All surgical procedures undertaken in both rats and dogs were non-recovery. At the end of each procedure rats were killed by the injection of a 2-3ml bolus of air i.v., followed by cervical dislocation. Dogs were killed with an overdose of barbiturate (Euthatal) administered i.v.. In both cases death was confirmed by monitoring heart rate, systemic blood pressure and respiration.

2.2.7(ii) Data Transformation.

The blood pressure pulse does not take the form of a sinusoidal wave as systole and diastole are not of equal length. As a consequence, mean arterial blood pressure cannot be derived by calculating the arithmetical mean of the systolic and diastolic blood pressure. A routinely used approximate of mean blood pressure is given by the following formula:

$$\text{Mean BP} = [(\text{Systolic BP} - \text{Diastolic BP}) \div 3] + \text{Diastolic BP}$$

This approximation of mean blood pressure was used in the present study.

Vascular resistance was calculated from blood flow and pressure which were measured directly. Resistance was calculated using the following formula:

$$\text{Resistance (dyne/s/cm}^3\text{)} = [\text{mean BP} \times 1333] \div \text{flow (ml/s)}$$

Change in minute flow and resistance was calculated using a Basic program shown in appendix (1).

2.2.7(iii) Statistical Analysis.

The Kolmogorov-Smirnov test was used to ascertain whether data was distributed in a "Normal" manner. This test compares the cumulative distribution function for a variable with a normal distribution. The Kolmogorov-Smirnov Z value is computed from the largest difference between the observed and theoretical distribution functions. A significantly large difference indicates that observed distribution function was not normally distributed.

When the sample size was large enough cumulative frequency was calculated and bar graphs plotted, normal curves were superimposed and the two compared. This was used in conjunction with the Kolmogorov-Smirnov test to investigate the normality of data.

An analysis of variance (ANOVA) was used to investigate the difference between treatment groups. This test indicates any significant variance between groups; follow-up tests were used to show where any differences lay. Tukey's honestly significant difference (HSD), Fisher's least significant difference (LSD) and Scheffe's test were used for this purpose. These tests all involve multiple comparisons between group means; the three tests were used to compensate for the type I and II errors associated with these procedures.

Blood pressure and heart rate data recorded from conscious normotensive and hypertensive rats was analysed by the Kolmogorov-Smirnov test and was plotted as a frequency histogram with a normal curve superimposed to ascertain the deviation from a normal distribution. An ANOVA with LSD, HSD and Scheffe's follow-up tests were performed on data from week I, when all animals received PEG alone. This was performed to

discover any differences between groups before atenolol treatment. The ANOVA and follow-ups were repeated using data from week IV, after 3 weeks atenolol administration to examine the differences following chronic treatment.

An unpaired t-test was used to compare two sample means by calculating Student's t and testing the significance of the difference between the means. This procedure was used if the data followed the normal distribution; if the data was non-parametric the Mann-Whitney U test was used.

In all experiments animals were randomly assigned to groups and drug treatments were randomly allotted to these groups. This procedure is a prerequisite for any meaningful statistical analysis.

All statistical analysis was carried out using the computer package SPSS-X version 2·1 on a Prime 9750 main-frame computer. Values of $p>0·05$ were taken as indicating no significant difference between the parameters under comparison.

2.2.7(iv) Drugs and general chemicals.

Noradrenaline bitartrate	Sigma Chemical Co. Inc.
Isoprenaline hydrochloride	Sigma Chemical Co. Inc.
Atenolol †	ICI Pharmaceuticals PLC
Sodium heparin	Duncan, Flockhart & Co
Sodium thiobutobarbitone (Inactin) †	BYK Gulden Lomberg Chem.
Sodium pentobarbitone (Sagatal)	May & Baker Ltd.
Sodium pentobarbitone (Euthatal)	May & Baker Ltd.
Desmethylimipramine hydrochloride.	Geigy Pharmaceuticals Ltd
L-(7,8) noradrenaline hydrochloride †	Amersham Int. PLC.
Polyethylene glycol (400 grade)	Fisons PLC.
Optiphase 'safe'	LKB Wallac Ltd.

† These drugs were kindly donated by ICI Pharmaceuticals PLC.

All doses of drugs given in the text are expressed as the weight of the salts. Drugs were prepared immediately before use, or were stored as frozen aliquots which were thawed as required. All drugs administered to conscious animals were given at room temperature.

2.3 RESULTS.

2.3.1 Results of the investigation of blood pressure.

2.3.1(i) Results from conscious normotensive Wistar rats.

During week I of the study, when all animals were dosed with Polyethylene glycol (PEG) alone, there was no significant difference between the two groups of animals. In both groups blood pressure was high on the first day of the treatment; this then stabilised at a lower level (around 152 mmHg).

"Active" drug treatment commenced at the beginning of week II and was continued for three weeks. At the start of week II, before treatment, the atenolol treated group had a mean systolic blood pressure of 153 (±1·3)mmHg while the control group had a mean blood pressure of 155 (1·7)mmHg. During the first week of atenolol treatment the mean systolic blood pressure of the treated group fell to a minimum of 131 (±3·2)mmHg while that of the control group remained relatively constant (approx 150mmHg). During this first week of dosing, a large difference between blood pressure recorded before and two hours after dosing was apparent in the treated group. Over the subsequent two weeks of dosing this difference was reduced. The blood pressure of the treated group stabilised at a reduced level over the second and third week of dosing. The mean blood pressure of the control group remained fairly constant over this period. Statistical analysis of the results obtained during week IV, the third week of dosing, showed a very highly significant difference between the treatment groups ($p<0·0001$). The

group treated with atenolol had a mean blood pressure over week IV of 140·6 (±1.5)mmHg while that of the control group was 153 (±1.6)mmHg.

These results are expressed graphically in figure 10 on page 75.

2.3.1(ii) Results from conscious spontaneously hypertensive rats.

There was no significant difference between the groups during week I when all animals received PEG alone. The systolic blood pressure of spontaneously hypertensive rats was found to be higher than that measured in normotensive animals. During control treatment with PEG hypertensive rats had a mean systolic blood pressures of 207·5 (±2·5) mmHg; while their normotensive Wistar counterparts had a mean sytolic blood pressure of 153·7 (±1·3) mmHg.

Dosing with atenolol commenced during week II, during this week the blood pressure of treated animals was dramatically reduced while that of the control group remained at its previous level. The variation in blood pressure measured before and after dosing was large during week I and diminished over time. This variation was negligible in control animals. Blood pressure remained reduced in treated animals over the whole dosing period. During week IV the mean systolic blood pressure in treated animals was 194 (±1.0)mmHg while that of control animals was 227 (±1.3)mmHg. This reduction in blood pressure was found to be very highly statistically significant ($p<0·0001$). The blood pressure was not not, however, reduced to levels found in normotensive rats. Figure 11 shows a graphical representation of these blood pressure results.

2.3.2 Results of the investigation of heart rate.

2.3.2(i) Results from conscious normotensive rats.

The graph of heart rate (figure 12) demonstrates that heart rate was variable during week I when both groups received PEG alone; there was no significant difference between groups. In general, heart rate was reduced during atenolol treatment from around 460 b/min to about 350 b/min over the first ten days of atenolol treatment. Heart rate is also reduced in the control group over the experimental period from around 460 b/min to around 400 b/min. Despite this, and the tendency for the heart rate of the treated animals to increase slightly, there was a significant difference between the groups ($p<0{\cdot}05$) during the final week of treatment.

2.3.2(ii) Results from conscious spontaneously hypertensive rats.

In the hypertensive rat, heart rate was seen to decrease in both groups over the entire experimental period. However, animals treated with atenolol exhibited a reduced heart rate compared with control animals. There was no statistically significant difference between groups during week I but after three weeks atenolol treatment the treated group exhibited a significantly reduced heart rate ($p<0{\cdot}0001$).

Figure 13 on page 78 is a graph of heart rate over the experimental period.

2.3.3 Results of the assessment of β-adrenoceptor blockade.

The dose response curve to isoprenaline was shifted to the right after 7 days pretreatment with atenolol indicating β-adrenoceptor blockade. The shift in the dose response curve was not, however, parallel. The displacement occurred in the upper portion of the curve and the maximum response appeared to be reduced. Figure 14 shows the dose response curves, it is apparent that there is no difference in the lower portion of the curves while there is a significant ($p<0{\cdot}01$) displacement in the upper portion.

A similar series of changes in dose response curves was observed after 21 days atenolol pretreatment (figure 15). The β-adrenoceptor blockade observed after 7 days pretreatment was still apparent after 21 days. The shift in the curves was limited to the upper portion of the curve as previously described. There was found to be a significant attenuation of the responses to the upper half of the isoprenaline dose response curve ($p<0{\cdot}01$) while no such difference was apparent in the lower portion of the curve.

2.3.4 Results from the *in-situ* blood perfused mesentery model.

2.3.4(i) Results from normotensive Wistar rats.

Pretreatment with atenolol for 7 days resulted in a tendency for an increase in response to higher doses of exogenous noradrenaline. This was not, however, very pronounced with the only significant increase occurring in response to a dose of 1000ng noradrenaline ($p<0{\cdot}05$). The dose response curves to exogenous noradrenaline following 7 days pretreatment are shown in figure 16.

Figure 17 shows the frequency response curves to periarterial electrical stimulation following 7 days atenolol pretreatment. The graph clearly shows that there is no difference in response between the control and the treated groups.

The dose response curves to exogenous noradrenaline following 21 days atenolol pretreatment are shown in figure 18. The dose response curves follow the same pattern as that previously described following 7 days pretreatment. There is some evidence of an increased mesenteric response to higher doses of noradrenaline. Although this is only statistically significant at a dose of 640ng ($p<0{\cdot}05$).

The mesenteric response to periarterial electrical stimulation following 21 days pretreatment with atenolol is significantly ($p<0{\cdot}05$) reduced at higher frequencies (16 & 35Hz). The graph of the frequency response curves is shown in figure 19.

The mean systemic blood pressure, mean mesenteric blood pressure and mesenteric resistance measured in the anaesthetised animals before responses to noradrenaline and electrical stimulation were ascertained are shown in appendix 2. There were no apparent differences in any of these parameters between animals pretreated with PEG or atenolol.

2.3.4(ii) Results from spontaneously hypertensive rats.

The dose response curves to exogenous noradrenaline shown in figure 20 are similar in shape to those obtained from normotensive animals. There was, however, an increased response to doses of exogenous noradrenaline in the spontaneously hypertensive animals.

There was a tendency for an increased response to higher doses of exogenous noradrenaline following 7 days atenolol pretreatment. The observed increase did not, however, reach statistical significance. This is a similar pattern of results to that observed in normotensive animals.

The frequency response curves shown in figure 21 are of a similar shape to those obtained from Wistar rats. A large increase in response compared with that of normotensive animals was, however, apparent over the whole stimulation frequency range.

There was no apparent change in response to periarterial electrical stimulation following 7 days atenolol pretreatment in the hypertensive rat, the two curves being almost superimposed; this was also true after 7 days treatment in normotensive animals.

The noradrenaline dose response curves obtained after 21 days pretreatment are shown in figure 22. The curve obtained from the treated animals was more sigmoidal in shape than the control curve. There was a significant ($p<0{\cdot}001$) attenuation of the middle portion of the curve while the lower and upper portions remain unchanged.

Figure 23 shows the stimulation frequency response curves after 21 days atenolol pretreatment. The curves are of a similar shape to the frequency response curves previously described. There is a very large parallel displacement of the curve obtained from the atenolol treated animals to the right. This attenuation of response to periarterial electrical stimulation is highly statistically significant ($p<0{\cdot}001$).

Data shown in appendix 2 shows that there was a tendency for an increase in "resting": mean systemic blood pressure, mean mesenteric blood pressure and mesenteric resistance following 7 days atenolol pretreatment in the anaesthetised rat. These parameters were, however, all reduced after 21 days atenolol pretreatment.

2.3.4(iii) Results from the investigation of ^{3}H-noradrenaline overflow.

The results from the investigation of tritiated noradrenaline overflow in the mesentery of anaesthetised Wistar rats are shown in the following tables.

Table 2: Results from untreated male Wistar rats.

	pre-stim. DPM	post-stim. DPM	overflow DPM
	2277·6	4047·6	1769·9
	3367·5	4498·3	1130·8
	3367·5	4377·7	1269·4
	3479·4	4526·4	1047·0
	3024·5	3834·5	810·0
mean	**3051·5**	**4256·9**	**1205·4**
SE	±188·2	±121·3	±142·8

Table 3: Results from Wistar rats following 21 days atenolol treatment.

	pre-stim. DPM	post-stim. DPM	overflow DPM
	5030·1	6270·1	1240·0
	3901·8	4295·1	393·3
	2799·1	3181·9	382·8
	1986·0	2216·7	230·7
	3719·1	4434·5	715·4
mean	**3487·2**	**4079·7**	**592·4**
SE	±461·9	±608·2	±161·1

There are no significant differences in the DPM values of samples taken before or after stimulation between control and treated animals. There is, however, a statistically significant ($p<0·05$) reduction in the overflow levels (difference between pre and post stimulation values) following pretreatment.

2.3.5 Results from the investigation of the effect of treatment on the mesenteric vascular responses of normotensive male Beagles.

2.3.5(i) Mesenteric blood pressure responses.

Figure 24 shows the mesenteric pressure dose response curve to exogenous noradrenaline. The dose response curves from the control and the treated animals are parallel and no significant difference was found between these curves.

The mesenteric pressure response to periarterial electrical stimulation is shown in figure 25. The curves from both treated and untreated animals are parallel. The responses obtained from the atenolol pretreated dogs are somewhat smaller than the control responses; this attenuation did not, however, reach statistical significance.

2.3.5(ii) Mesenteric blood flow responses.

The mesenteric flow response to exogenous noradrenaline illustrated in figure 26 shows the distribution of the flow responses to doses of noradrenaline. The computer generated regression lines show that the % decrease in flow is directly proportional to the dose of noradrenaline. This is true of both the control and treated responses; the two regression

lines were found to be not significantly different. Responses to individual doses of exogenous noradrenaline were also found to exhibit no significant differences between treated and control groups.

There was also no statistically significant differences between the treated and control groups in respect to the mesenteric flow response to electrical stimulation. This is shown in the graph on page 92 (figure 27).

2.3.5(iii) Mesenteric resistance response.

Figure 28 shows the mesenteric response to exogenous noradrenaline in terms of resistance. There is no significant difference between the curve obtained from the untreated control animals and that obtained from dogs pretreated with atenolol.

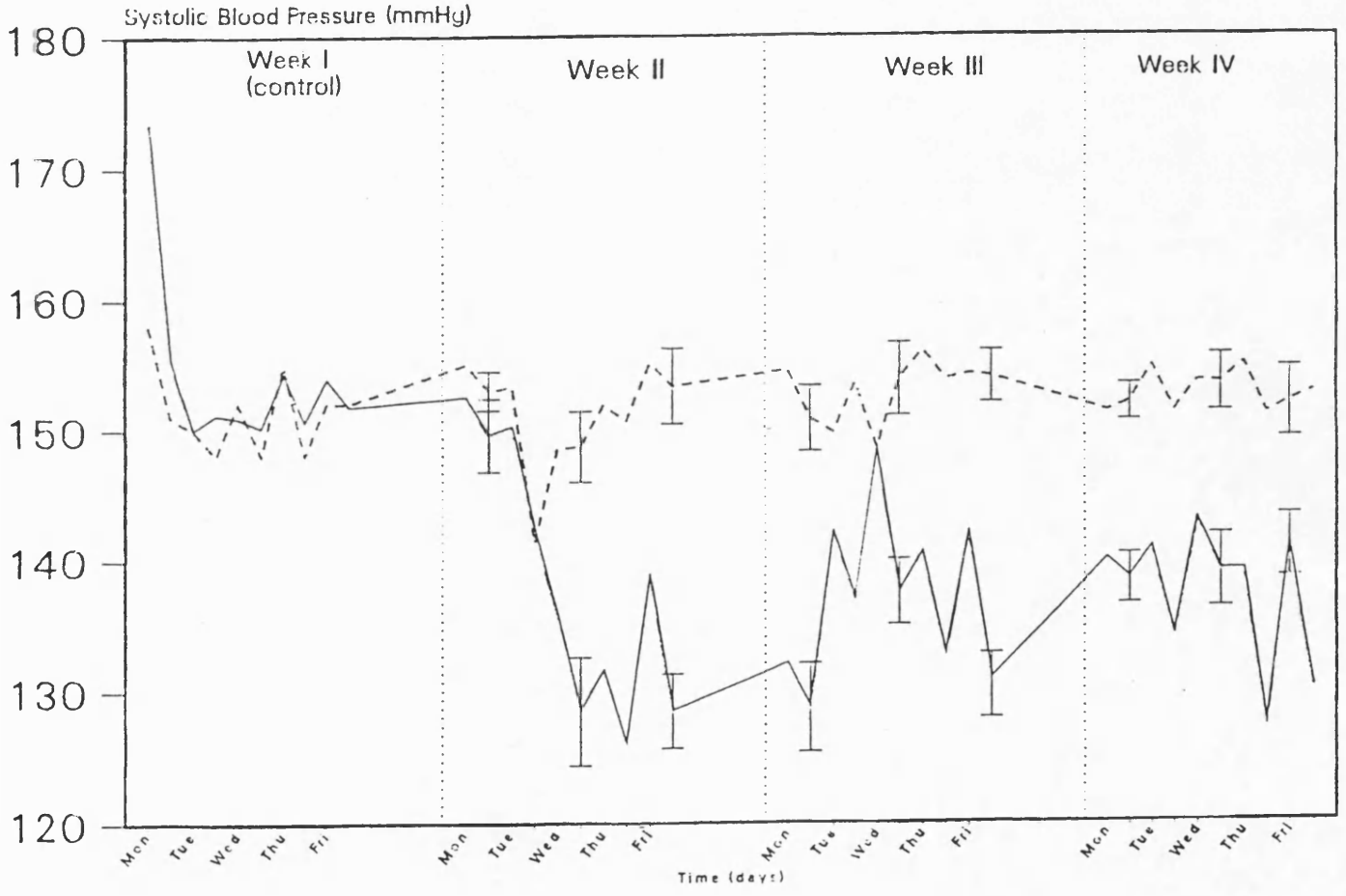
The graph on page 94, figure 29, shows the mesenteric resistance response to periarterial electrical stimulation. There is a marked attenuation of the mesenteric resistance response to periarterial electrical stimulation. This reduction is statistically significant ($p<0{\cdot}05$) at every stimulation frequency except 16Hz.

In the following graphs (figures 10-29), statistical difference was calculated between the values from control animals (treated with PEG) and the corresponding values from atenolol treated animals. This difference is shown by the following notation :

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE 10

Graph showing the effect of atenolol (50mg/kg) p.o. on systolic blood pressure in the conscious normotensive male Wistar rat.

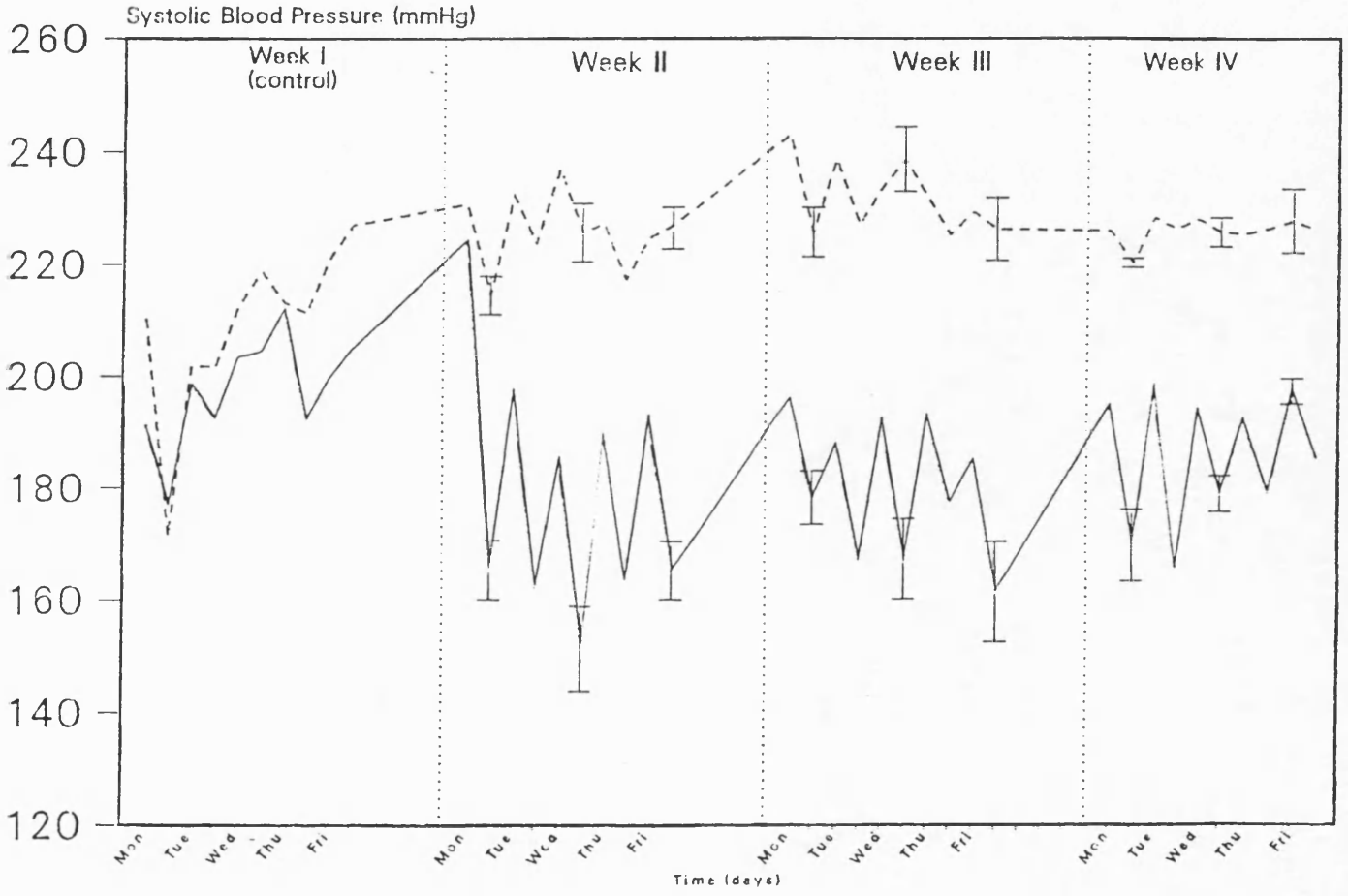


——— Atenolol (50mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=8.

----- Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=6.

FIGURE 11

Graph showing the effect of atenolol (50mg/kg) p.o. on systolic blood pressure in the conscious spontaneously hypertensive male Japanese Okamoto rat.

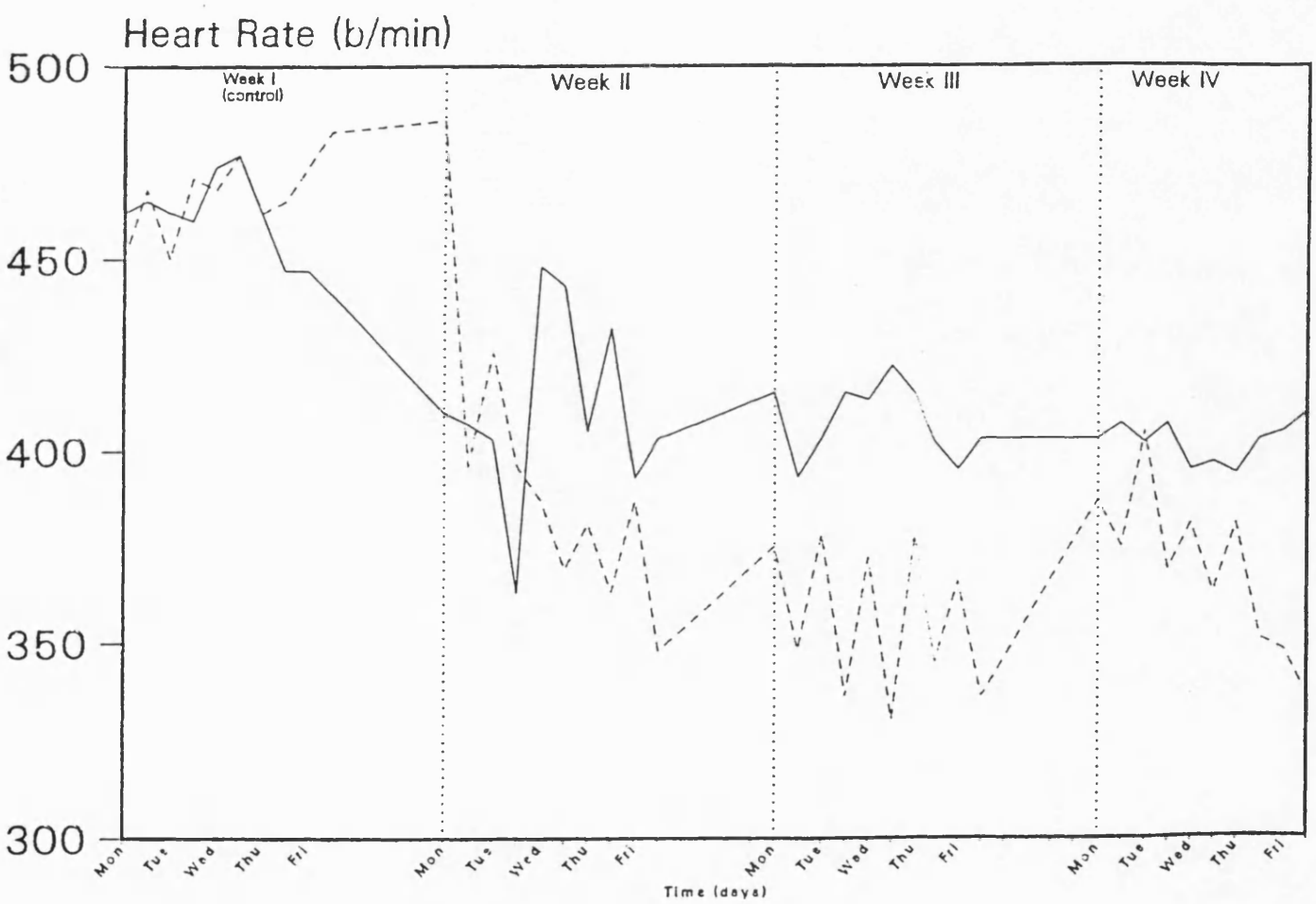


——— Atenolol (50mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=4.

----- Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=4.

FIGURE 12

Graph showing the effect of atenolol (50mg/kg) p.o. on heart rate in the conscious normotensive male Wistar rat.

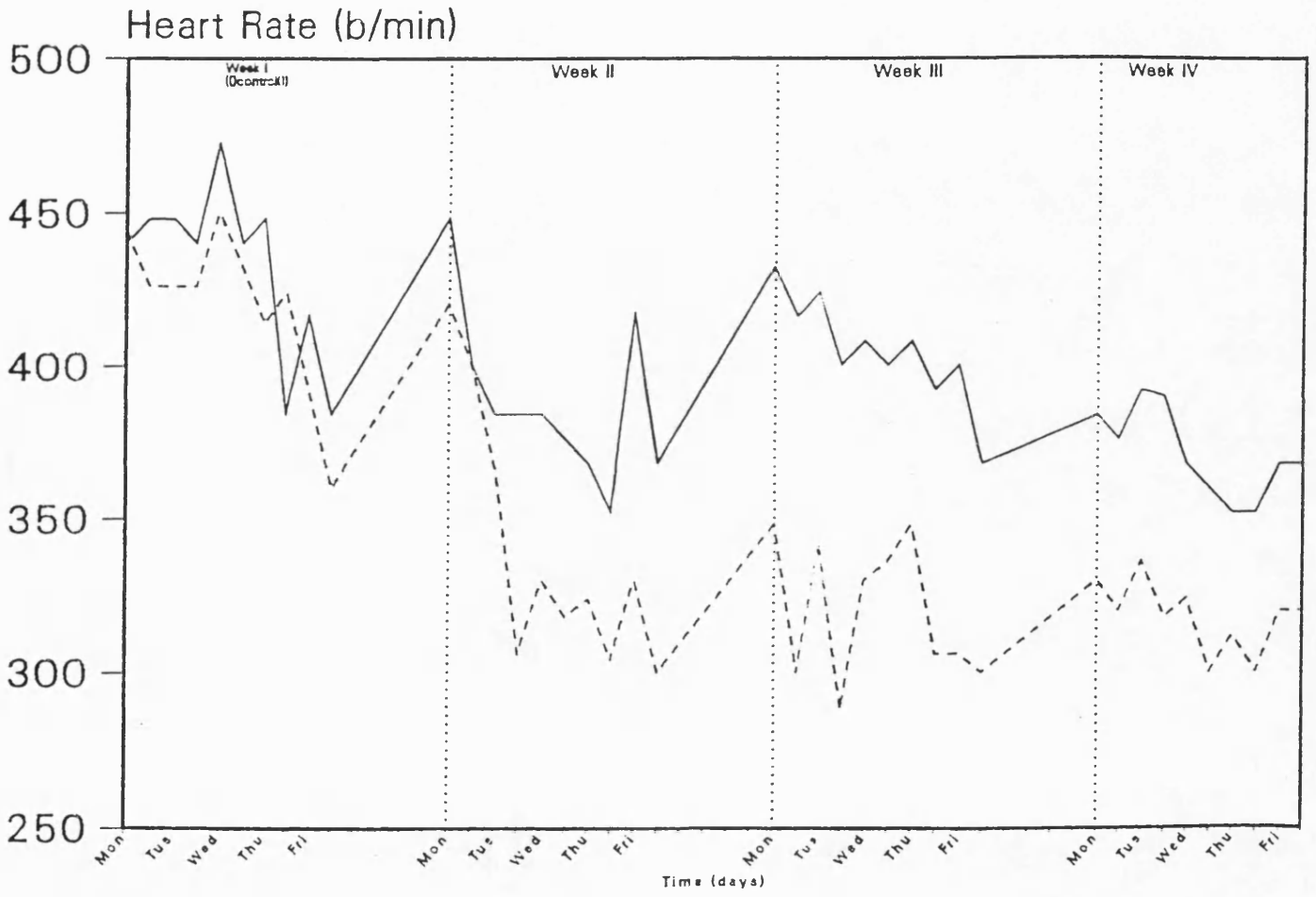


----- Atenolol (50mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=8.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=6.

FIGURE 13

Graph showing the effect of atenolol (50mg/kg) p.o. on heart rate in the conscious spontaneously hypertensive male Japanese Okamoto rat.

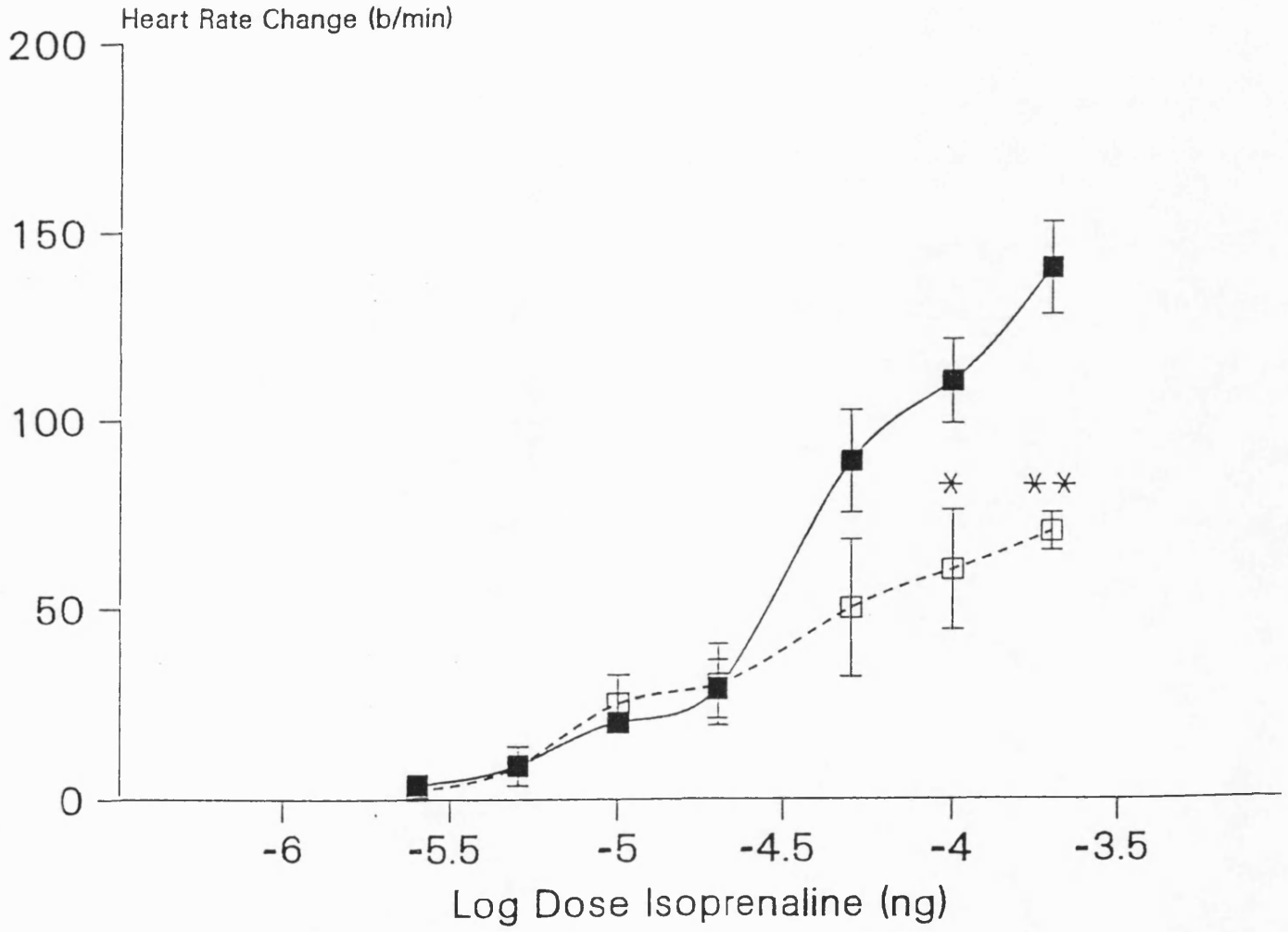


----- Atenolol (50mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=4.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=4.

FIGURE 14

Graph showing the effect of 7 days pretreatment with atenolol (50mg/kg) p.o. on the heart rate response to exogenous isoprenaline in the anaesthetised Wistar rat.



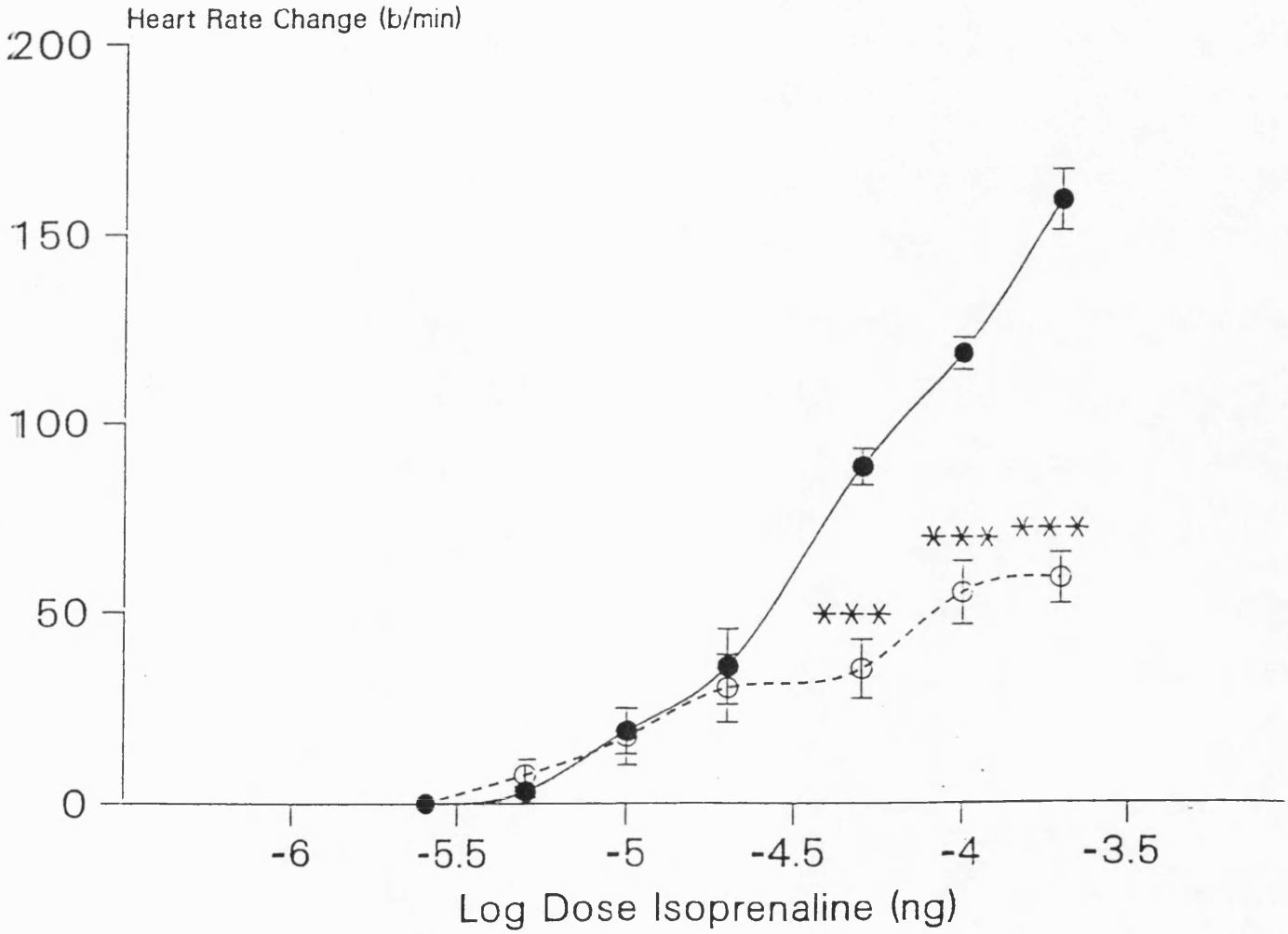
□----□ Atenolol (50mg/kg) p.o. (n=4) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=8) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 15

Graph showing the effect of 21 days pretreatment with atenolol (50mg/kg) p.o. on the heart rate response to exogenous isoprenaline in the anaesthetised Wistar rat.



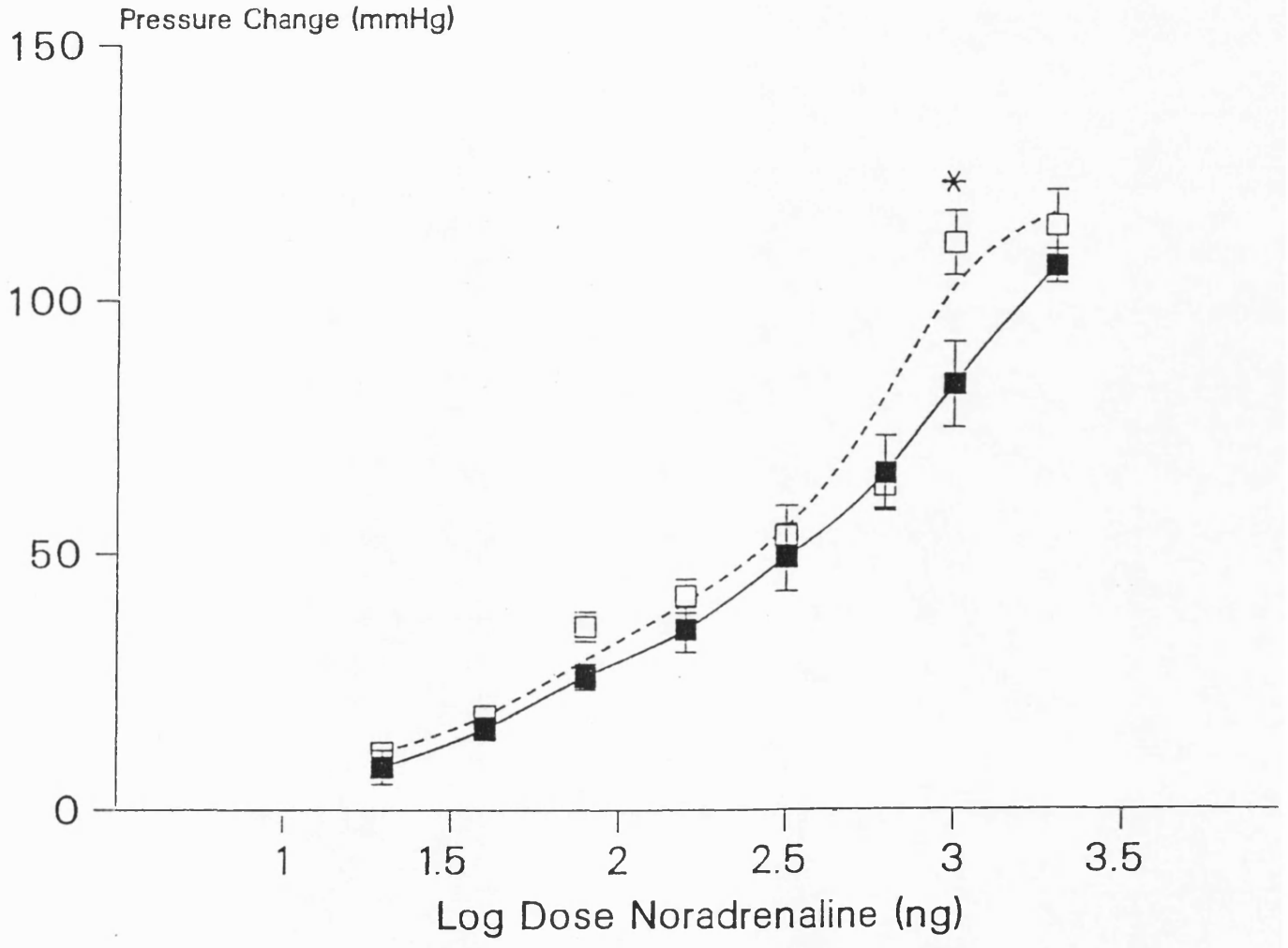
o----o Atenolol (50mg/kg) p.o. (n=4) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=8) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 16

Graph showing the effect of 7 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Wistar rat.



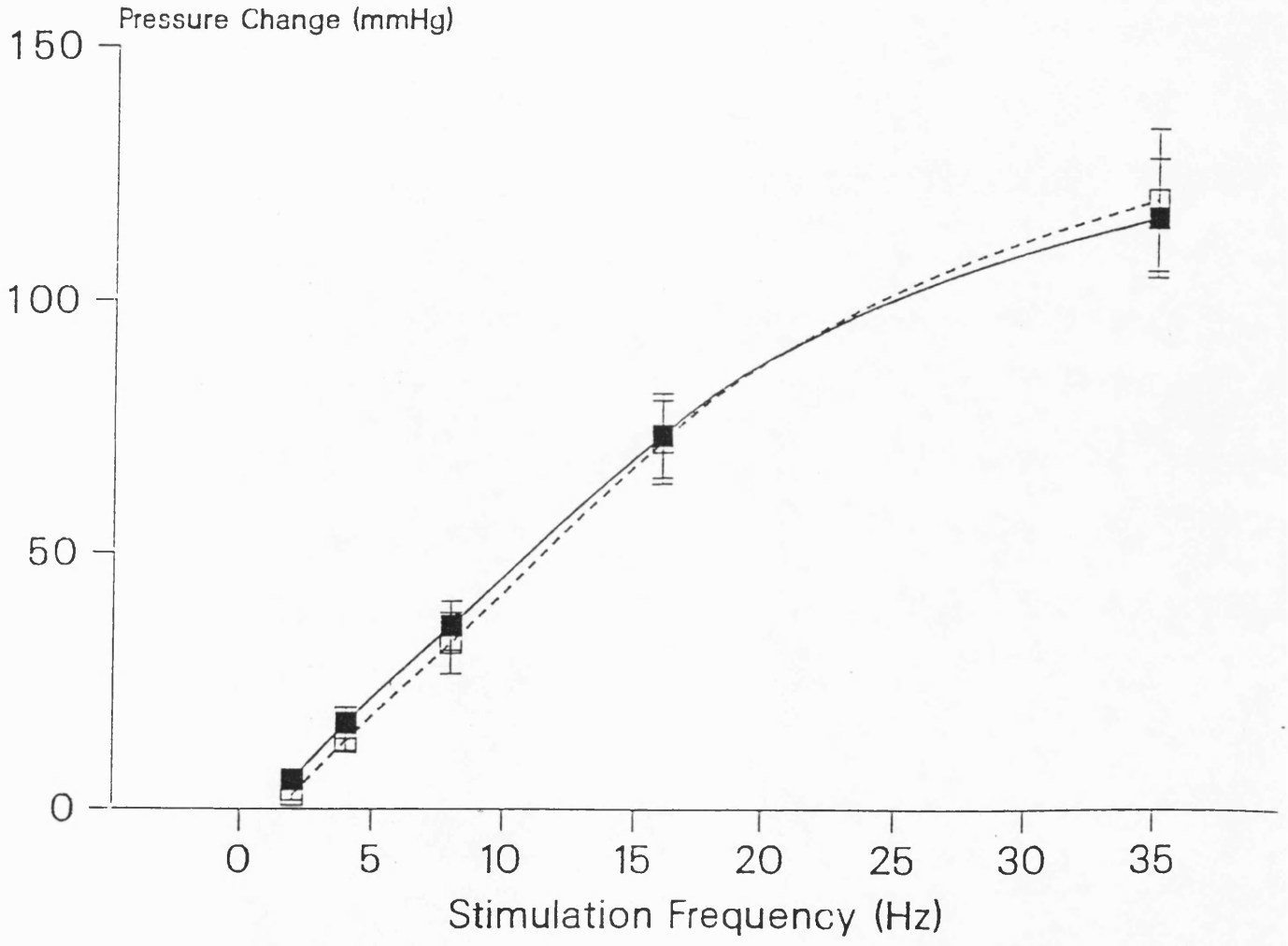
□----□ Atenolol (50mg/kg) p.o. (n=10) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 17

Graph showing the effect of 7 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Wistar rat.

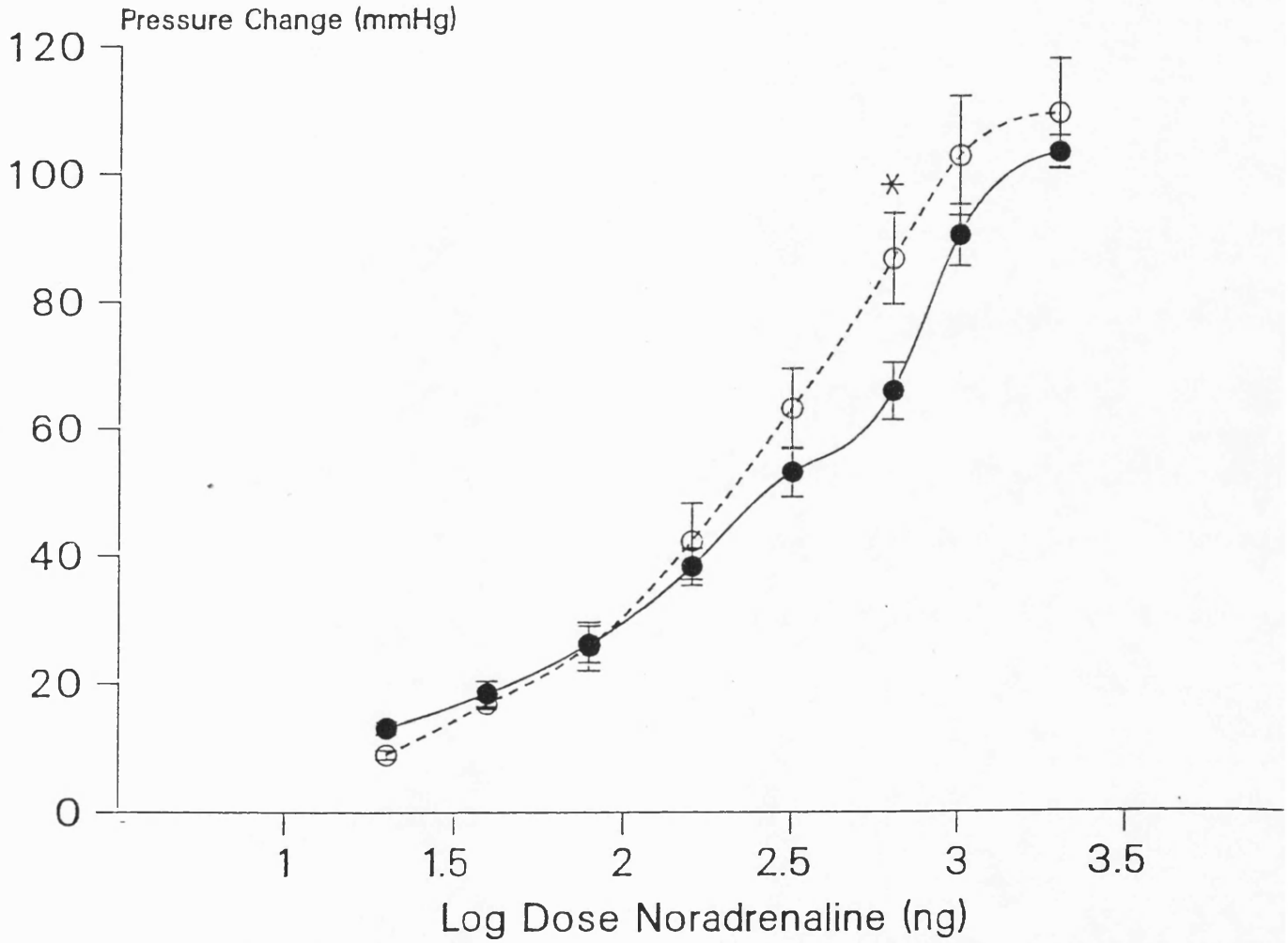


□----□ Atenolol (50mg/kg) p.o. (n=10) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 18

Graph showing the effect of 21 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Wistar rat.



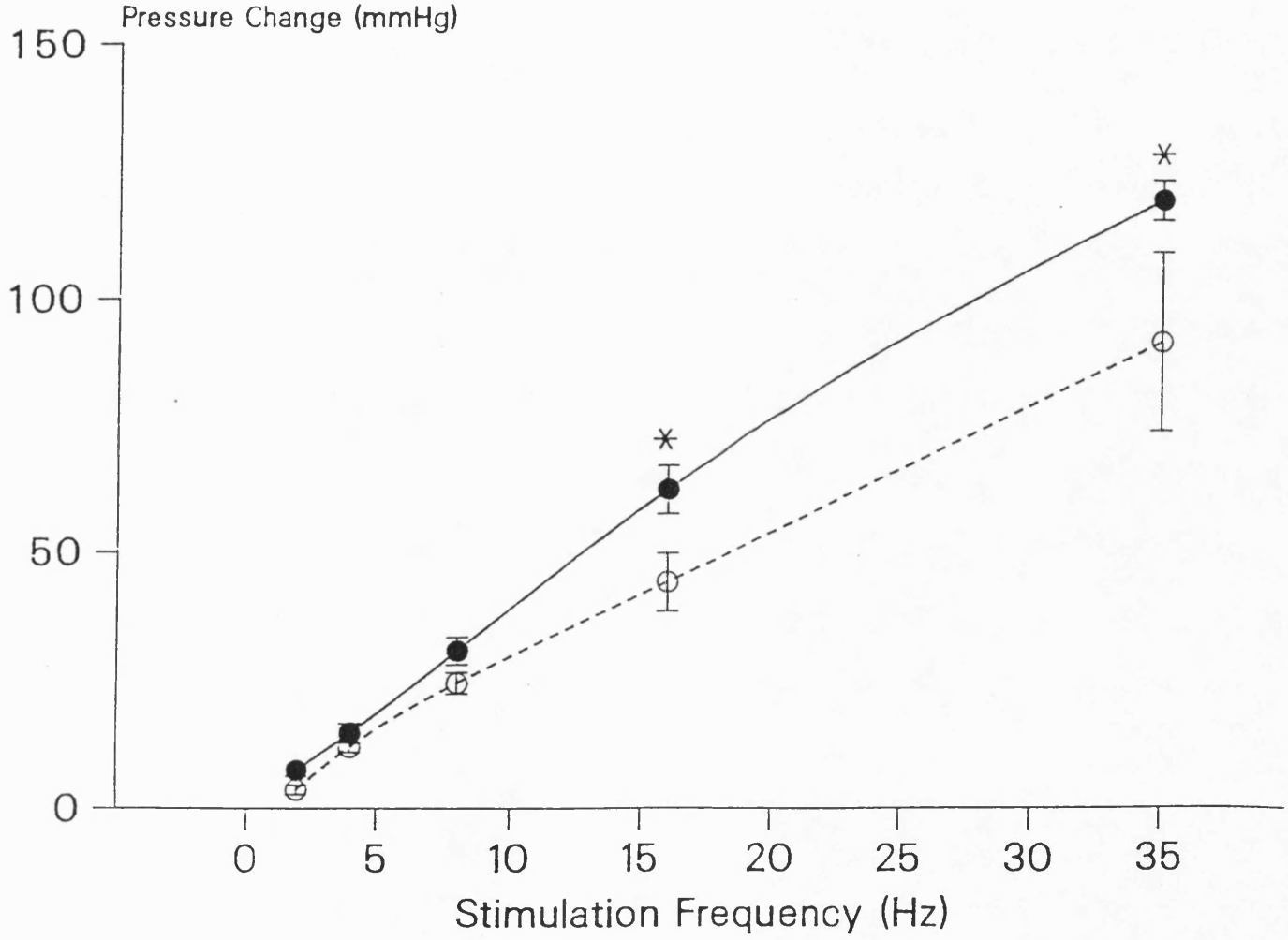
o----o Atenolol (50mg/kg) p.o. (n=7) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=7) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 19

Graph showing the effect of 21 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Wistar rat.



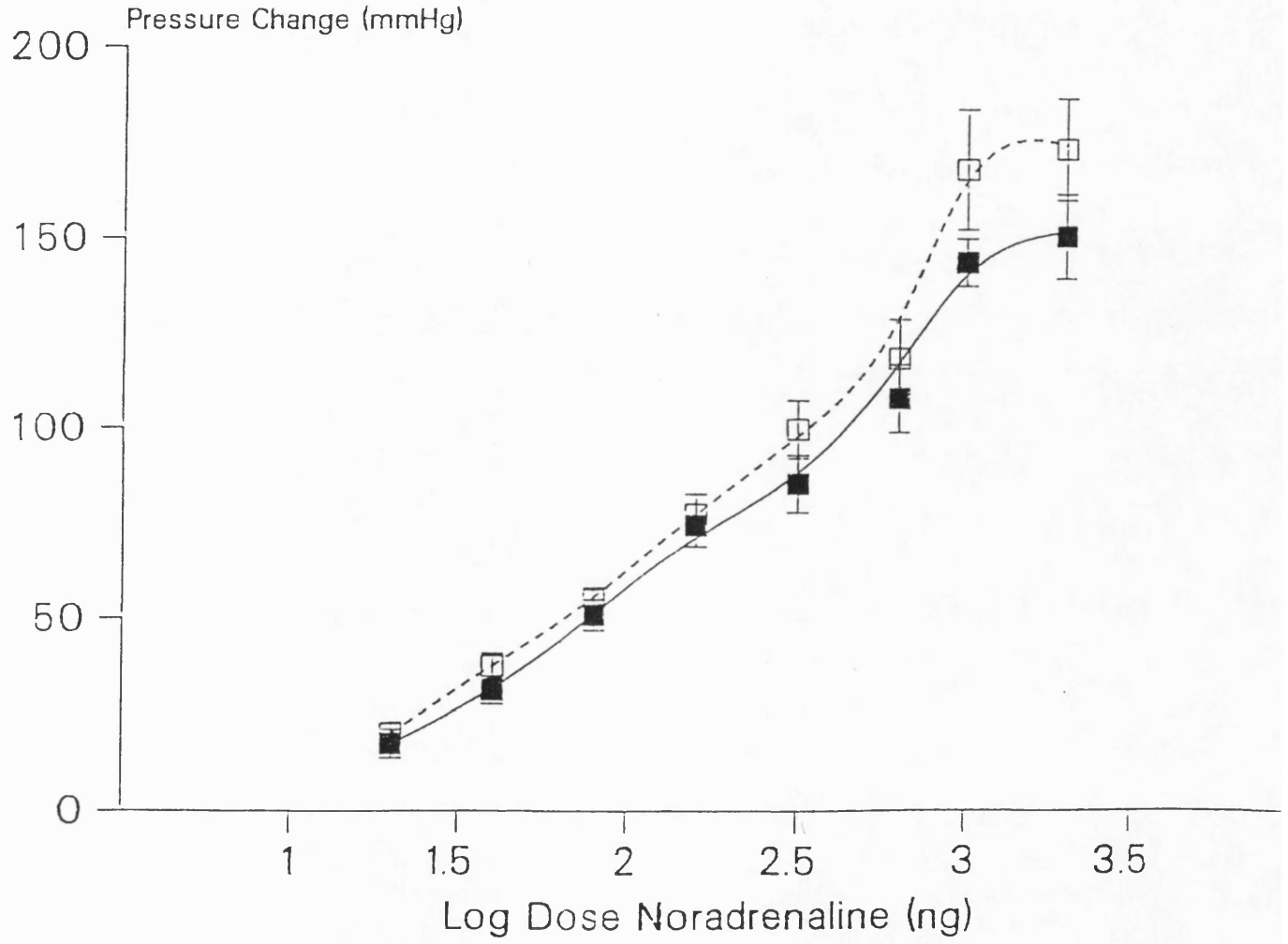
o----o Atenolol (50mg/kg) p.o. (n=7) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=7) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 20

Graph showing the effect of 7 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Japanese Okamoto SHR.

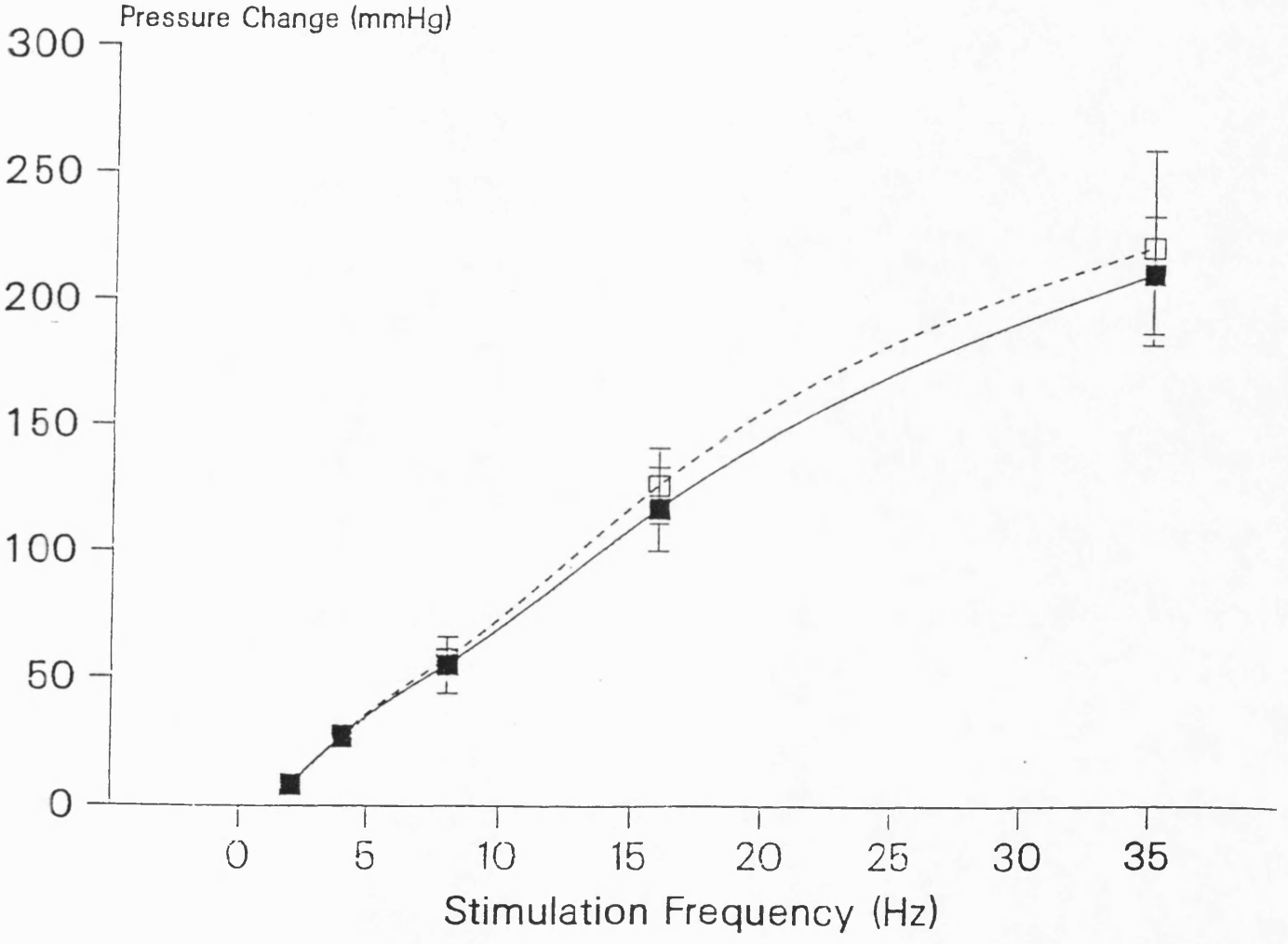


□----□ Atenolol (50mg/kg) p.o. (n=6) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 21

Graph showing the effect of 7 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Japanese Okamoto SHR.

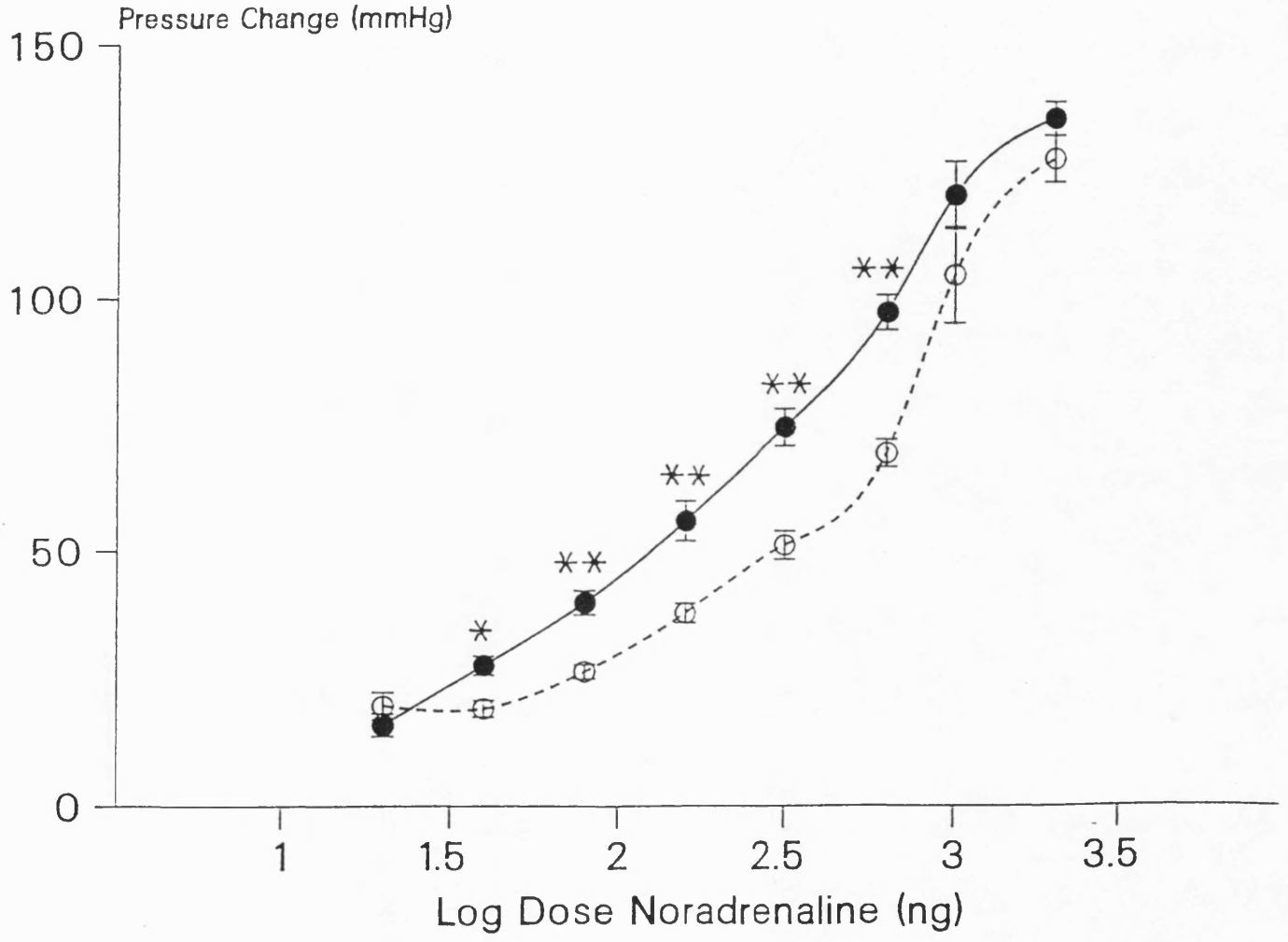


□----□ Atenolol (50mg/kg) p.o. (n=6) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=5) mean ±SE.

FIGURE 22

Graph showing the effect of 21 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Japanese Okamoto SHR.

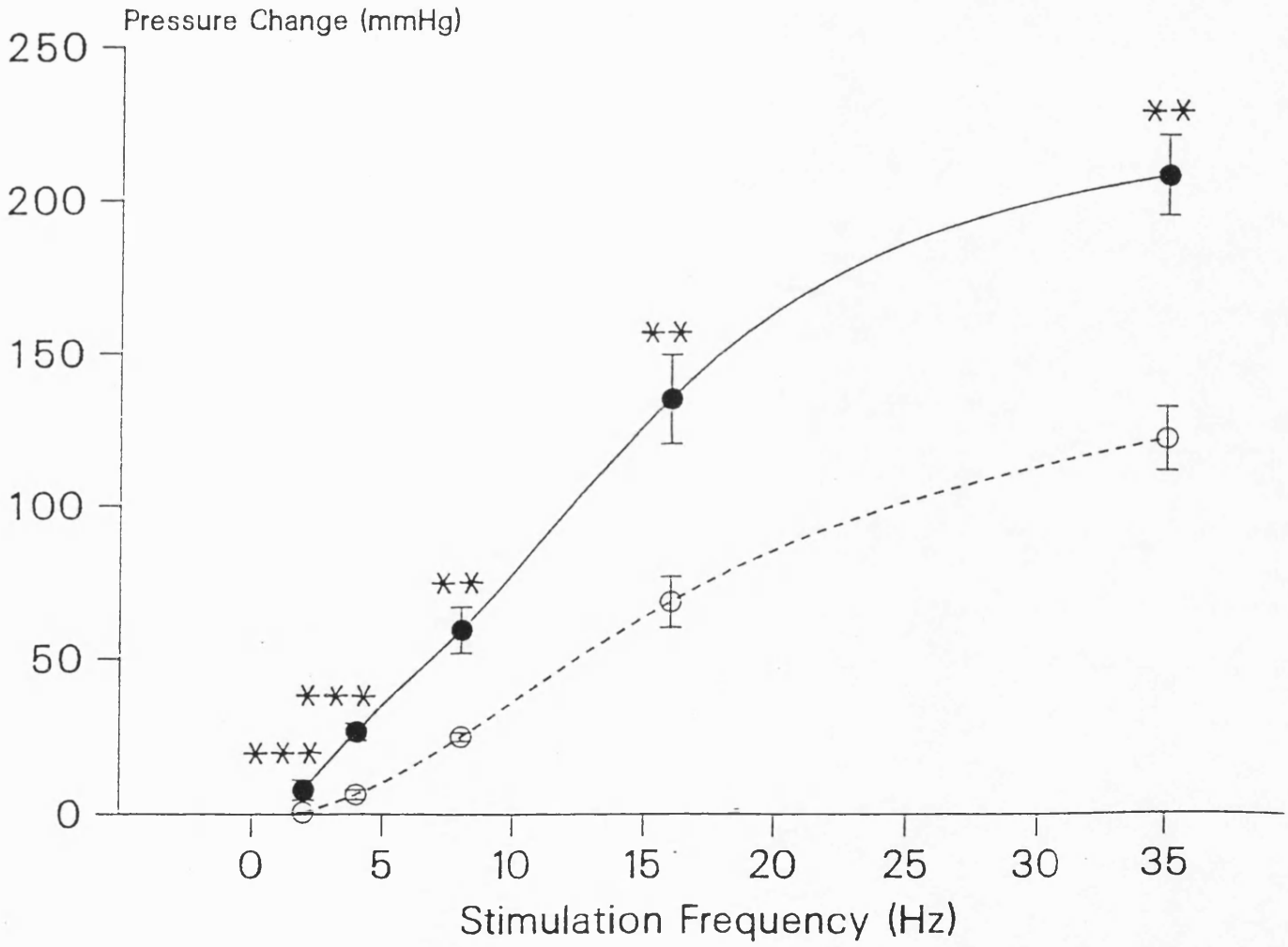


o----o Atenolol (50mg/kg) p.o. (n=7) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 23

Graph showing the effect of 21 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Japanese Okamoto SHR.



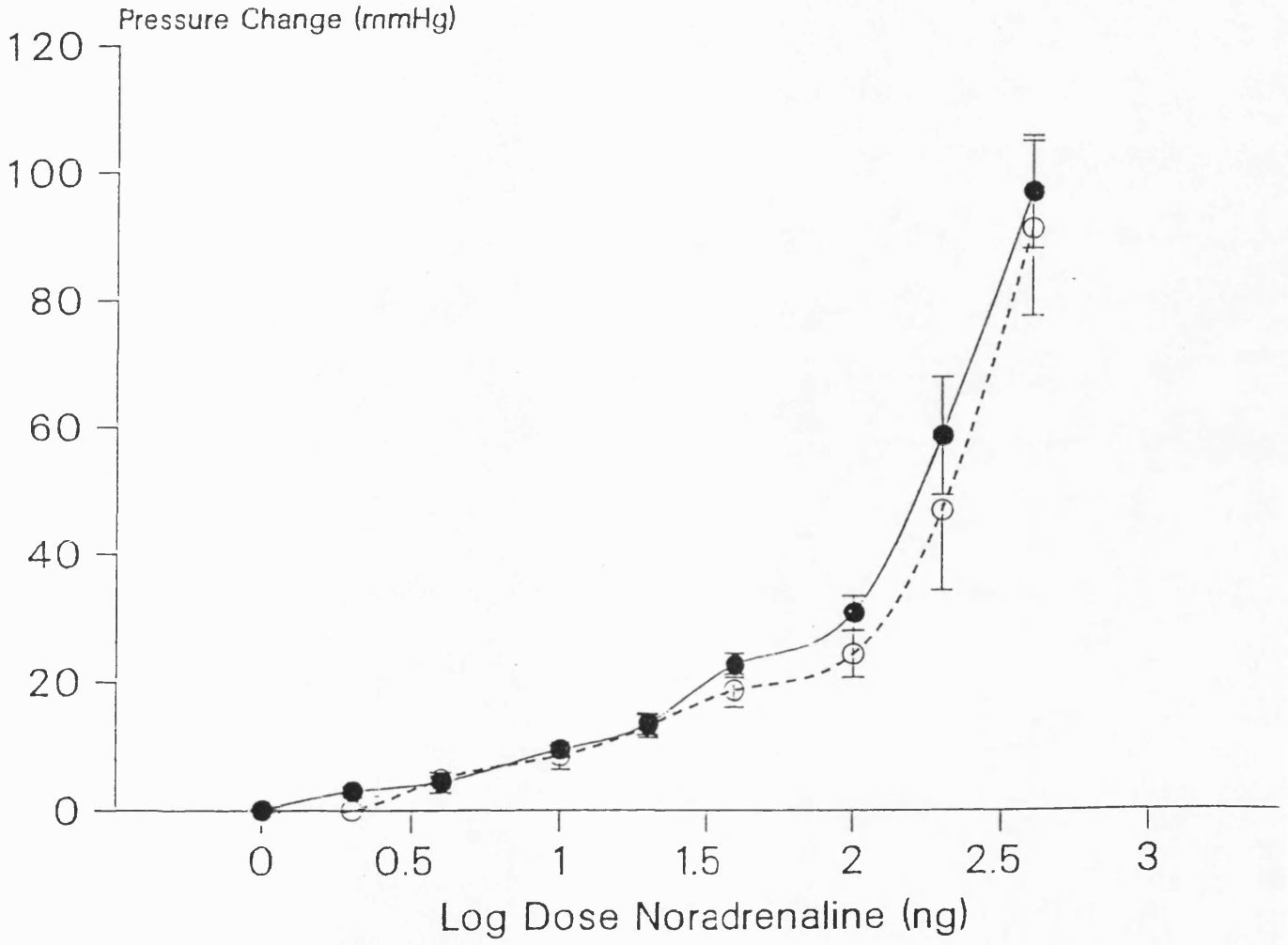
o----o Atenolol (50mg/kg) p.o. (n=7) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 24

Graph showing the effect of 21 days pretreatment with atenolol (7·5mg/kg) p.o. on the mesenteric blood pressure response to exogenous noradrenaline in the anaesthetised Alderly Park Beagle.

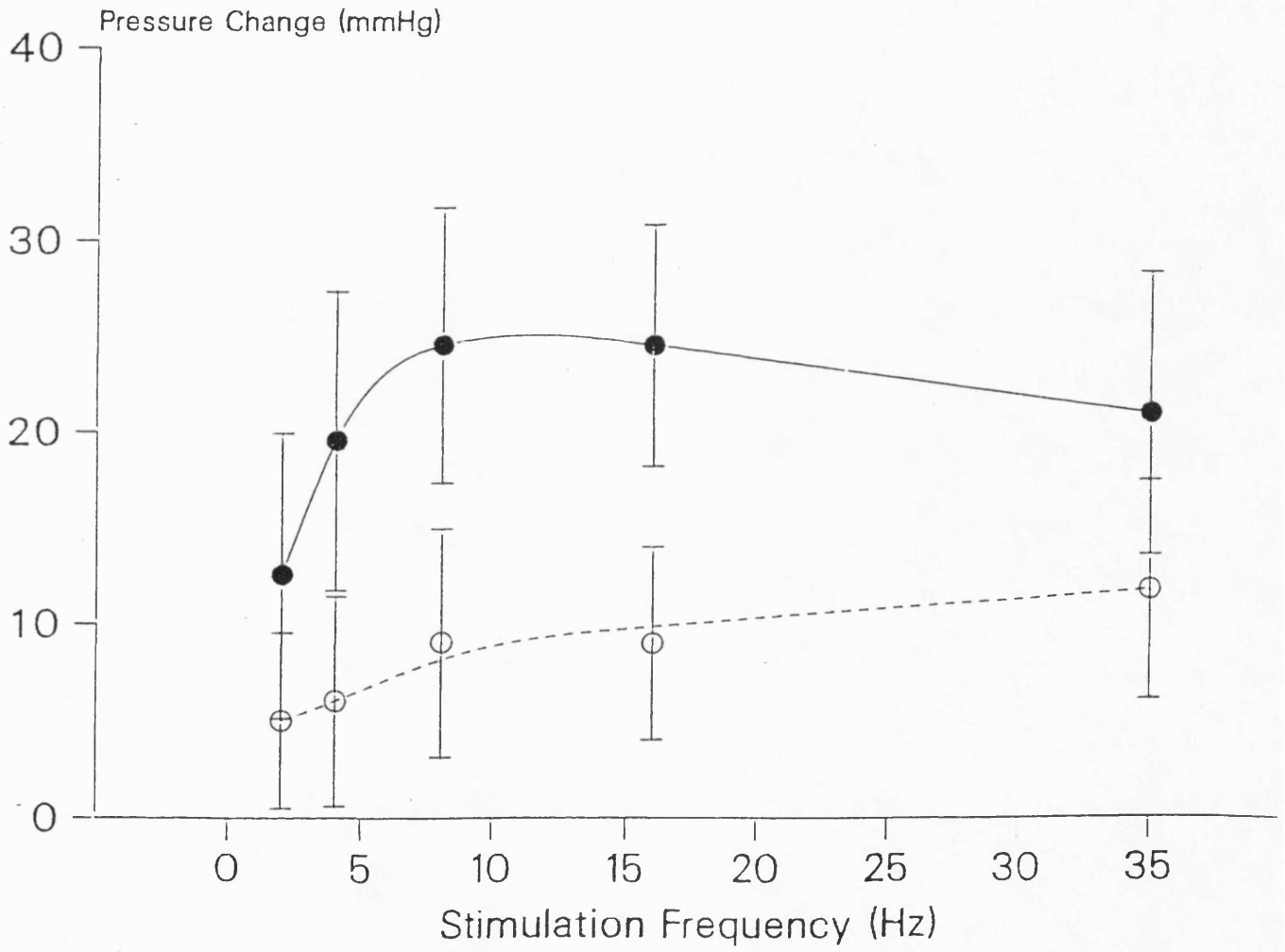


o----o Atenolol (7·5mg/kg) p.o. (n=5) mean ±SE.

●——● Untreated control. (n=7) mean ±SE.

FIGURE 25

Graph showing the effect of 21 days pretreatment with atenolol (7·5mg/kg) p.o. on the mesenteric blood pressure response to periarterial electrical stimulation in the anaesthetised Alderly Park Beagle.

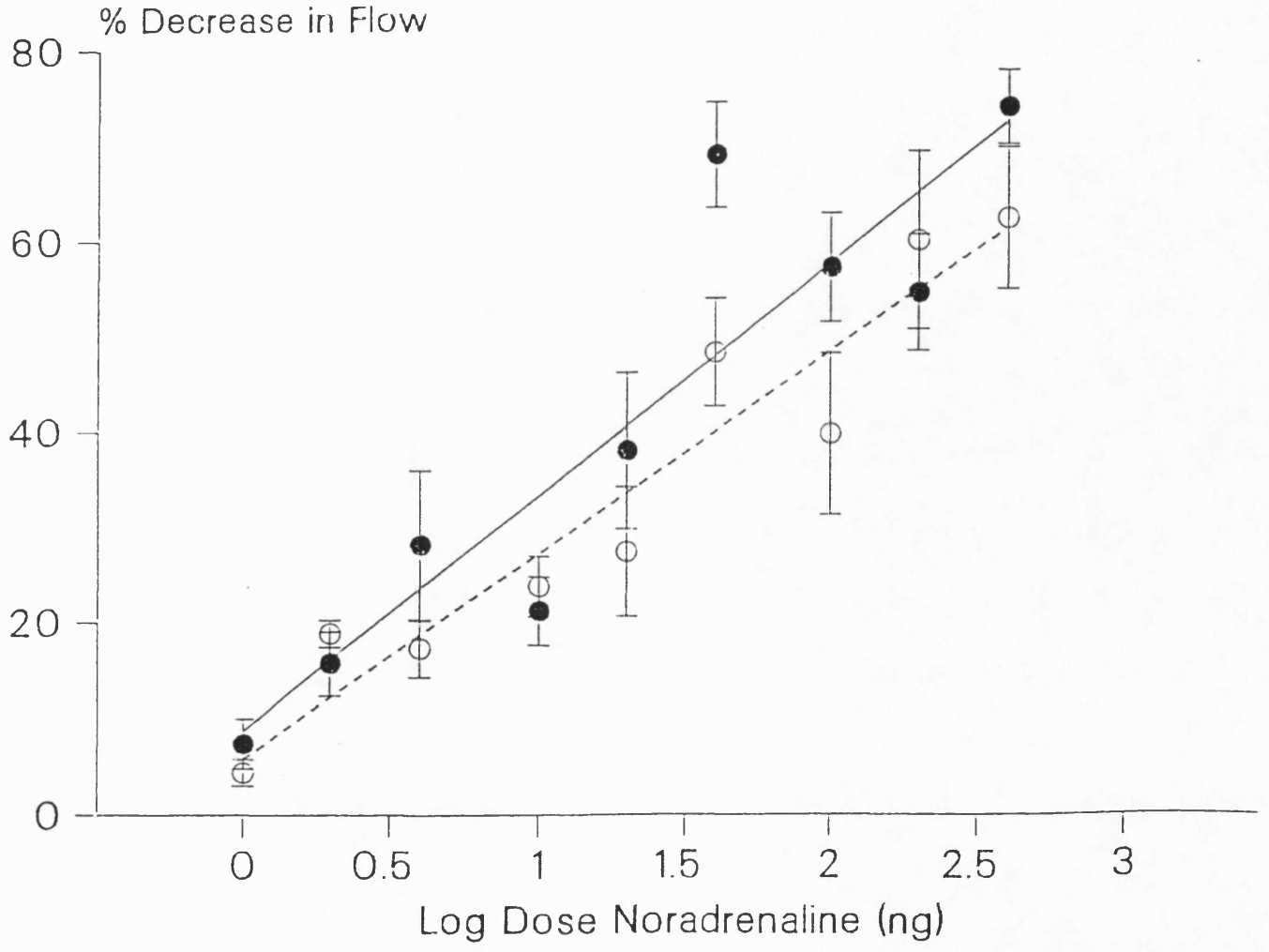


o----o Atenolol (7·5mg/kg) p.o. (n=5) mean ±SE.

●——● Untreated control. (n=7) mean ±SE.

FIGURE 26

Graph showing the effect of 21 days pretreatment with atenolol (7·5mg/kg) p.o. on the mesenteric blood flow response to exogenous noradrenaline in the anaesthetised Alderly Park Beagle.

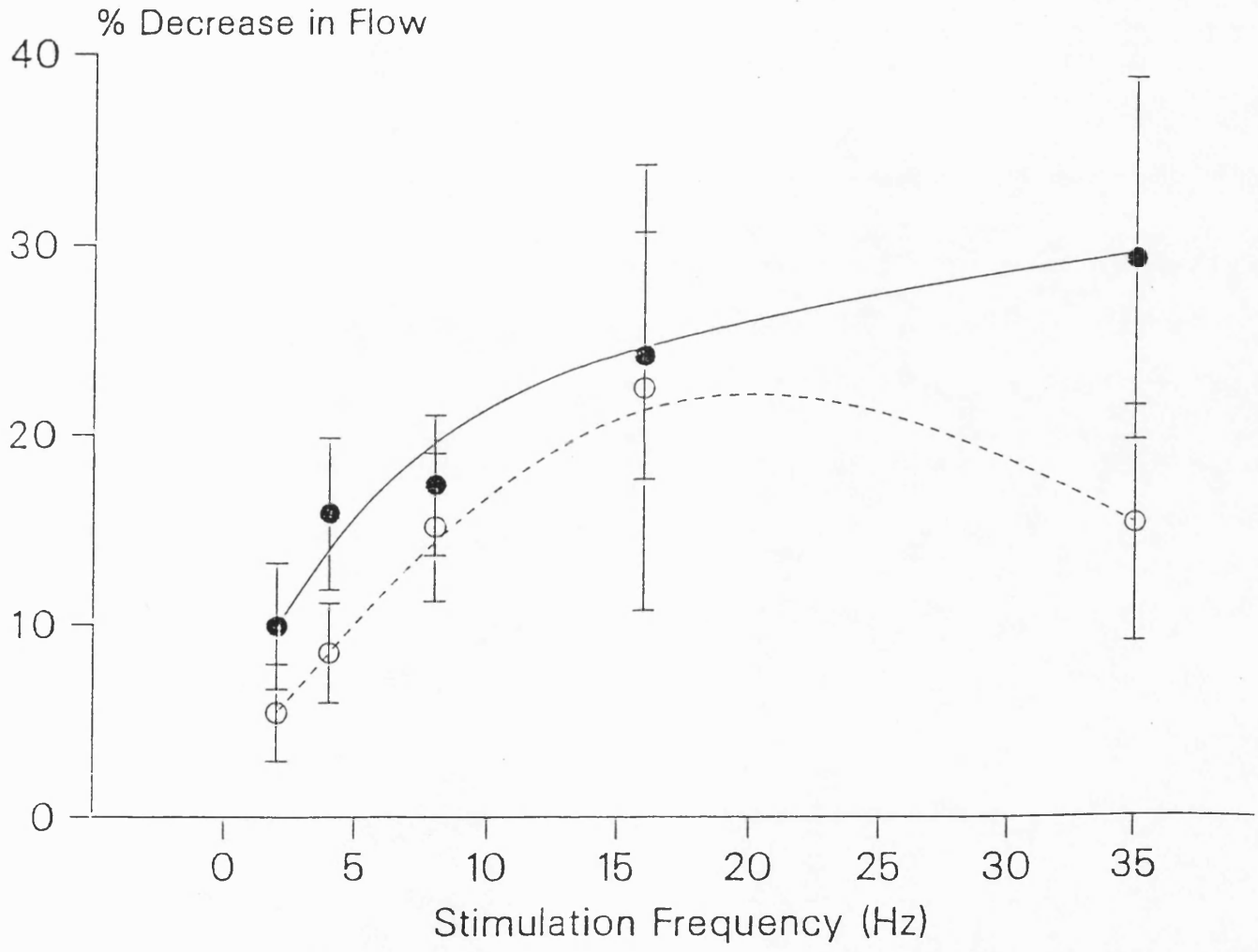


o----o Atenolol (7·5mg/kg) p.o. (n=5) mean ±SE.

●——● Untreated control. (n=7) mean ±SE.

FIGURE 27

Graph showing the effect of 21 days pretreatment with atenolol (7·5mg/kg) p.o. on the mesenteric blood flow response to periarterial electrical stimulation in the anaesthetised Alderly Park Beagle.

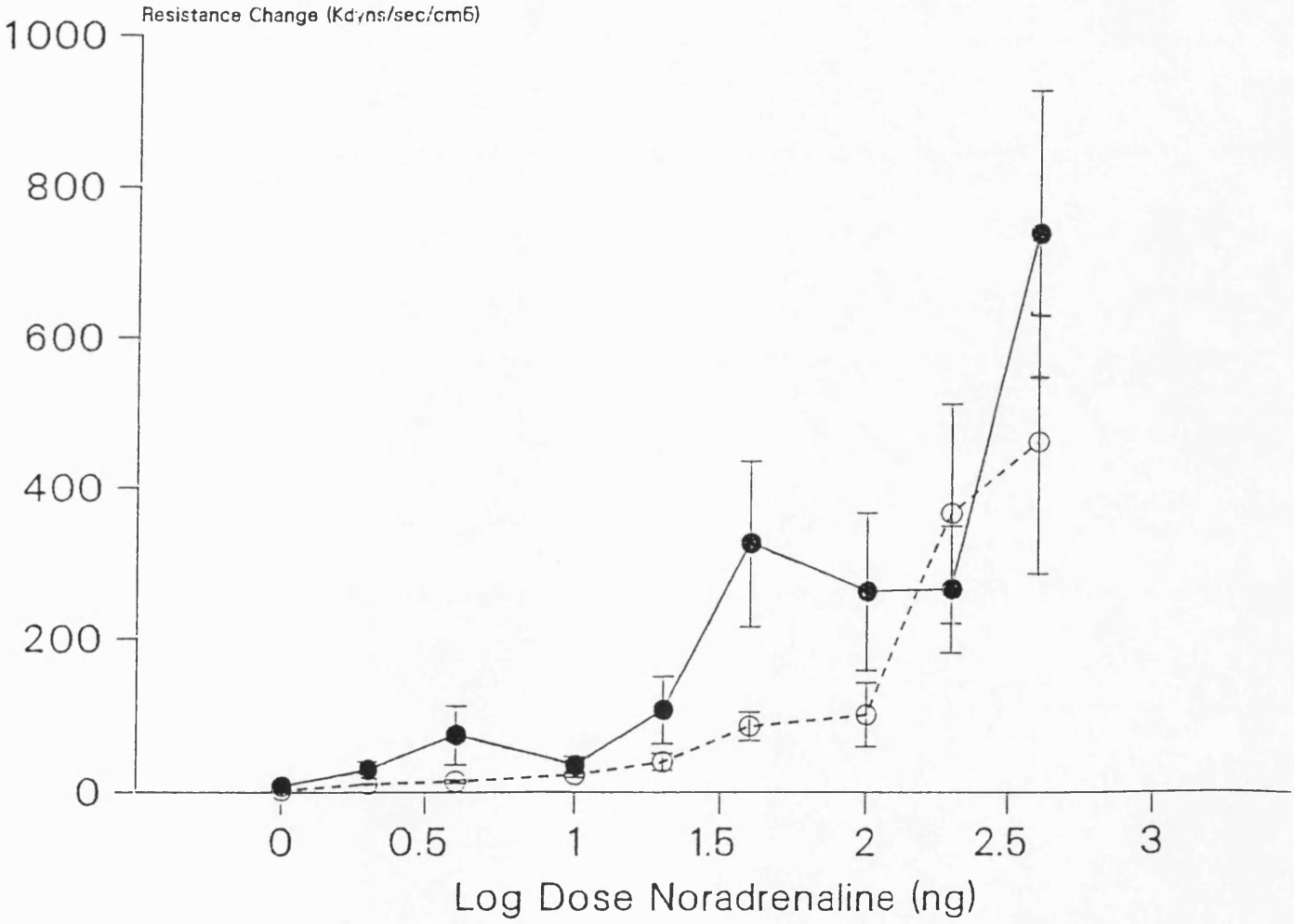


o----o Atenolol (7·5mg/kg) p.o. (n=5) mean ±SE.

●——● Untreated control. (n=7) mean ±SE.

FIGURE 28

Graph showing the effect of 21 days pretreatment with atenolol (7·5mg/kg) p.o. on the mesenteric vascular resistance response to exogenous noradrenaline in the anaesthetised Alderly Park Beagle.

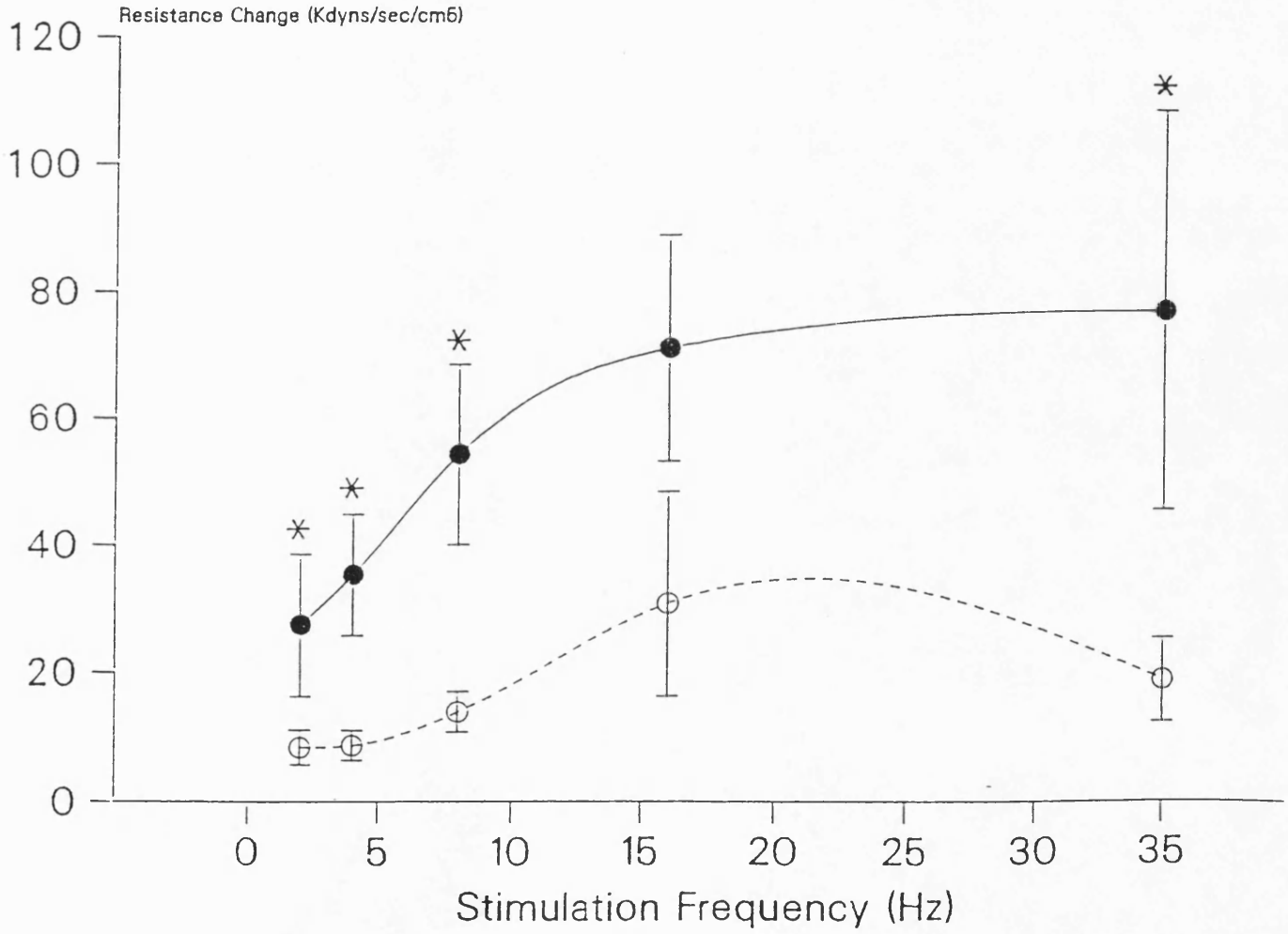


o----o Atenolol (7·5mg/kg) p.o. (n=5) mean ±SE.

●——● Untreated control. (n=7) mean ±SE.

FIGURE 29

Graph showing the effect of 21 days pretreatment with atenolol (7·5mg/kg) p.o. on the mesenteric vascular resistance response to periarterial electrical stimulation in the anaesthetised Alderly Park Beagle.



o----o Atenolol (7·5mg/kg) p.o. (n=5) mean ±SE.

●——● Untreated control. (n=7) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

2.4 CONCLUSIONS.

2.4.1 Conclusions from the conscious rat studies.

Treatment of conscious normotensive and spontaneously hypertensive rats with atenolol (50mg/kg p.o.) reduced both heart rate and systolic blood pressure. This can clearly be seen in figures 10-13 inclusive (pages 75-78).

Results are consistent with the theory that hypertension is a result of raised peripheral resistance rather than an increase in cardiac output. It was apparent during treatment with the control that the systolic blood pressure of the conscious hypertensive rats was significantly higher (around 35%) than that of their normotensive counterparts. There was, however, no significant difference in heart rate between normotensive and hypertensive animals.

Atenolol given orally in the manner previously described (section 2.2.1) reduced both the heart rate and blood pressure of rats. The whole time course of the decrease in blood pressure was interesting. Pressure fell to a minimum over the first week of atenolol treatment and then reached a plateau at a slightly higher level over the subsequent two weeks of dosing. This was apparent in both normotensive and hypertensive animals but was clearer in the normotensive group. This raised the possibility of change in the hypotensive mechanism(s) of action of atenolol over the dosing period. Also the variation in blood pressure recorded 2 and 24 hours after each dose of atenolol decreased over the experimental period. This variation was especially noticeable in hypertensive animals. The

daily troughs in blood pressure (2 hours after dosing) coincide closely with expected daily peak plasma levels of the drug (see section 2.1.5). The reduction in this variation may suggest, that with chronic administration, atenolol possibly reached concentrations that produced a more constant hypotensive action.

The decrease in blood pressure observed in hypertensive animals was greater than that in normotensives, although it was not reduced to normotensive levels. This larger reduction may be as a result of the higher initial pressure, which provided more scope for reduction. Alternatively it is possible that atenolol acted by "correcting" a pathophysiologically abnormal blood pressure control system and thus helped to normalise blood pressure. As the same system could be expected to be "normal" in normotensive animals there would be less of a reduction in blood pressure.

While the changes in heart rate, shown by both normotensive and hypertensive animals, over the treatment period follow a similar pattern to that of blood pressure, there are some important differences. The blood pressure of the normotensive Wistar rats did not fall significantly until the second day of dosing, while the heart rate was reduced immediately. Also, in general, changes in blood pressure did not "mirror" changes in heart rate, that is, the peaks and troughs of blood pressure did not match those in heart rate. This evidence supports the hypothesis that atenolol's hypotensive action is distinct from the acute decrease in cardiac output.

2.4.2 Conclusions from the assessment of β-adrenoceptor blockade.

The dose response curve obtained in response to isoprenaline was shifted to the right after both 7 and 21 days pretreatment with atenolol (figures 14 and 15). This shift in dose response curve is indicative of β-adrenoceptor blockade and suggests that blockade is present after both 7 and 21 days atenolol pretreatment. The shift in the dose response curve was not parallel; displacement occurred in the upper portion and the maximum appeared to be reduced. The shift and shape of the curves was similar after both 7 and 21 days pretreatment. The shape of the isoprenaline curves following atenolol pretreatment were not as would be expected for competitive β-adrenoceptor antagonism. This is probably largely due to the experimental method used to investigate it. The response to isoprenaline was measured by the β_1-mediated increase in heart rate, which is antagonised by atenolol. Heart rate in the anaesthetised animal can, however, be influenced by other systems which may be affected by isoprenaline's β_2-mediated actions which are not antagonised by atenolol. The change in the balance of isoprenaline's β_1 and β_2-adrenoceptor mediated action may thus explain the shape of the dose response curve following atenolol pretreatment.

The situation is further complicated by methodological difficulties encountered with this technique. The range of heart rate changes was such that differences in the changes produced by low doses of isoprenaline may not have been apparent due to the scale employed in recording. This would make the lower portions of the dose response curves appear closer than was actually the case. Also, high doses of isoprenaline caused death in anaesthetised animals due to the combined effect of its β_2-mediated vasculature vasodilation which results in a reduction in cardiac return and

its tendency to cause arrhythmias. This resulted in difficulty in obtaining a maximal heart rate response to isoprenaline. Additionally it is harder to obtain the maximal increase in rate in pretreated animals, as death, due to isoprenaline's "unblocked" β_2-mediated actions, occurs before the dose is high enough to produce a maximum response in terms of its attenuated β_1-mediated effects. This effect may result in distortion of the dose response curve and the combined effect of these methodological problems may explain the shape of the dose response curves obtained.

These methodological difficulties could have been avoided by using a Langendorff heart preparation to assess β-blockade. This, or other isolated preparations would have produced results that were more straightforward and easier to analyse. They would, however, contravene the initial aim of undertaking the work *in-situ* as far as possible.

2.4.3 Conclusions from the *in-situ* blood perfused mesentery model.

The results from the investigation of adrenergic neurotransmission using this *in-situ* blood perfused model were interesting (see section 2.3.4 and figures 16-23).

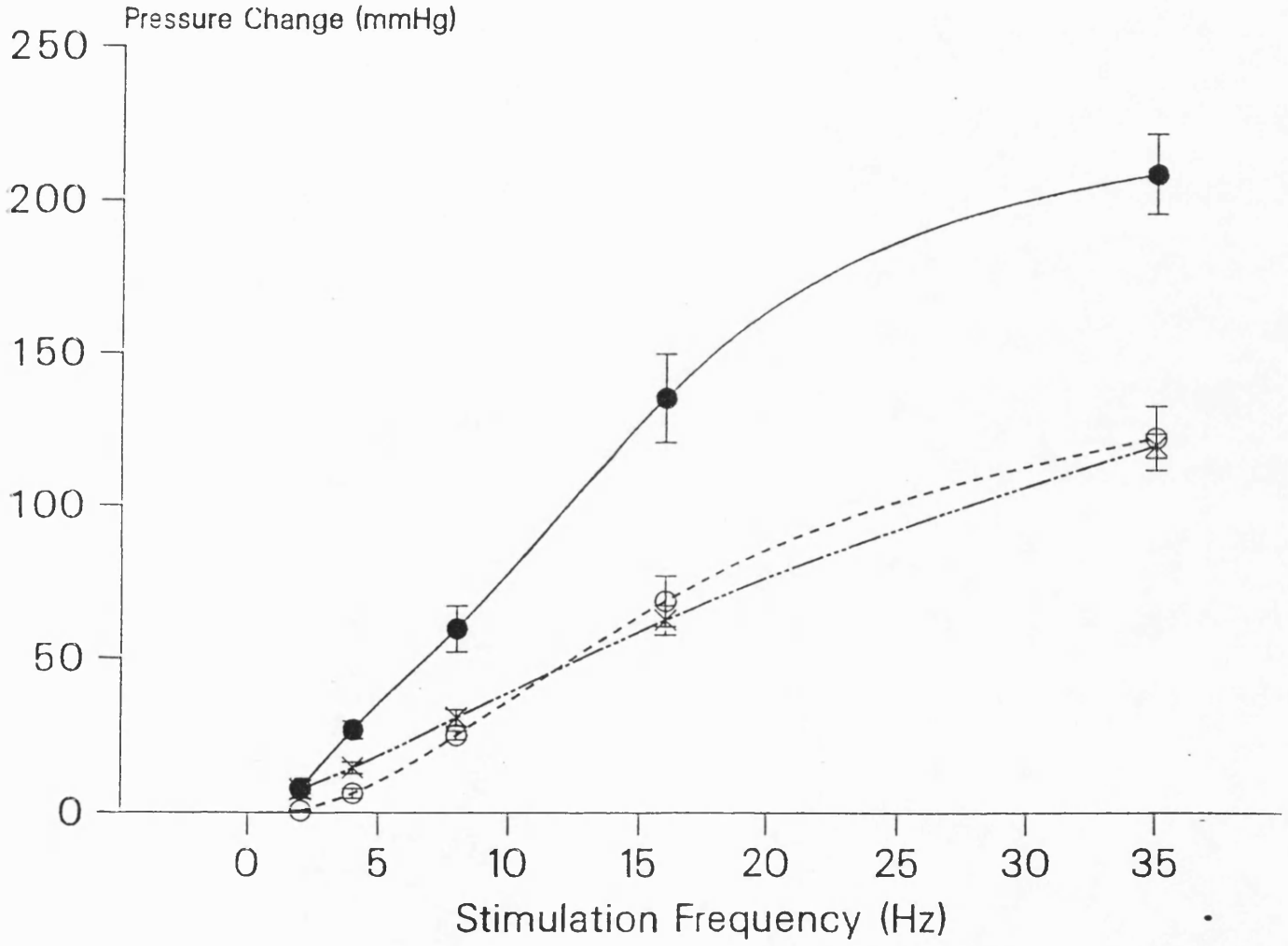
The results show that the spontaneously hypertensive rats treated with the PEG control were more sensitive to both exogenous noradrenaline and periarterial electrical stimulation than their normotensive counterparts. This supports the numerous studies that suggest that there is an increase in noradrenergic neurotransmission in the spontaneously hypertensive rat (Lefevre-Borg *et al* 1988, Yamamoto & Cline 1987, Westfall & Meldrum 1985).

Pretreatment of normotensive rats with atenolol for 7 days produced little change in response apart from a tendency for an increased response to higher doses of noradrenaline. This tendency was still apparent after 21 days pretreatment and more importantly was combined with a significant reduction in response to periarterial electrical stimulation. This suggests that, after 21 days pretreatment with atenolol, changes in adrenergic neurotransmission have occurred which result in a reduced response to stimulation. As the response to exogenous noradrenaline has not been greatly altered it is possible that this is the result of some presynaptic effect of atenolol.

The effect of 7 days pretreatment in spontaneously hypertensive rats is similar to that in normotensives. After 21 days, however, there is a reduction in response to exogenous noradrenaline in the mid part of the dose response curve and a very large reduction in response to electrical stimulation. The response to electrical stimulation following 21 days atenolol pretreatment in the hypertensive has been reduced to the control level found in normotensive animals. This is clearly shown in the graph on the following page (Figure 58). Once again this strongly suggests that after 21 days atenolol is exerting some presynaptic effect to reduce adrenergic neurotransmission. The size of this effect is interesting, as it seems that noradrenergic neurotransmission, which has been shown to be increased in hypertension (Lefevre-Borg *et al* 1988) has been reduced to normotensive levels (see figure 58). The time course of the effect is also interesting since it is unrelated to β-blockade, which has been shown to be present after 7 days; suggesting it may be involved in the plateau effect of blood pressure reduction observed in the conscious animals. The size and time course of this effect also suggests that it may be important in the antihypertensive effect of atenolol observed clinically.

FIGURE 58

Graph showing the effect of 21 days pretreatment with atenolol (50mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised rat.



o----o Atenolol (50mg/kg) p.o. Hypertensive rat (n=7) mean ±SE.

●——● PEG p.o. Hypertensive rat (n=6) mean ±SE.

X···X PEG p.o. Normotensive rat (n=7) mean ±SE.

Results of experiments pre-loading the mesentery with ^{3}H-noradrenaline supported the theory that atenolol was having some chronic presynaptic effect independent of its β-blockade. There was evidence that 21 days pretreatment with atenolol in normotensive rats caused a significant reduction in ^{3}H-noradrenaline overflow. This suggests that the chronic effect of atenolol on adrenergic neurotransmission involves a reduction in transmitter release. Lack of time precluded extending this work to include other pretreatment times and hypertensive animals. It is interesting to speculate that this effect may not be present after 7 days atenolol pretreatment and may be larger in hypertensive animals. Although there is no evidence to support this, it would fit with the pattern of previous work with the *in-situ* blood perfused mesentery.

2.4.4 Conclusions from the investigation of mesenteric vascular responses in normotensive male Beagles.

The lack of an atenolol effect on the mesenteric response to exogenous noradrenaline combined with a reduced response to periarterial electrical stimulation suggests that chronic atenolol may have a presynaptic effect in the dog. This would support and complement the work previously described in the rat.

The mesenteric response recorded in the normotensive dog is interesting (Figures 24-29). The method of investigation was different from that used in the rat (section 2.2.6) and allowed the mesenteric blood pressure, flow and resistance to vary (unlike in the rat where flow was kept constant). While there was no obvious change in response to noradrenaline, the change observed to electrical stimulation was more

clearly apparent when resistance was calculated. As in this preparation the animals were able to vary both flow and pressure in response to stimulation, changes were more apparent when both these parameters were used to calculate resistance. This raises the possibility that a similar method involving variable flow and pressure in the rat may have been both more physiological and more sensitive. Unfortunately limitations in the size and availability of the flow probes prevented such a method being investigated.

2.4.5 Overall conclusions.

Atenolol (50mg/kg p.o.) has been shown to reduce both blood pressure and heart rate in normotensive and hypertensive rats. The hypotensive effect was bigger in hypertensive animals and appeared to be separate from acute β-blockade. The antihypertensive effect of atenolol appeared to change over time, with a minimum value after 7 days treatment and a subsequent slight increase to a plateau.

Investigation of the effects of atenolol on adrenergic neurotransmission has shown that atenolol has a presynaptic effect reducing transmitter release. This effect has been demonstrated in both normotensive and hypertensive rats and in dogs. The putative presynaptic effect is not apparent after 7 days atenolol treatment but is clearly seen after 21 days. This presynaptic effect is distinct from acute β-adrenoceptor blockade; and its size and time course suggest that it may be important in atenolol's clinically observed antihypertensive effect.

These conclusions are more fully discussed and compared with those from other treatment groups in chapter 5.

CHAPTER 3.

NITRENDIPINE

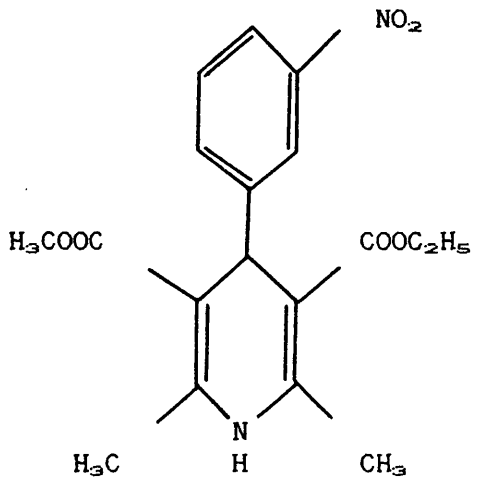
3.1 INTRODUCTION.

3.1.1 Nitrendipine: a historical overview.

Tanshinone, a naturally occurring calcium antagonist, has featured in Chinese cardiovascular medicine for three thousand years (Nayler & Dillon 1986). Current interest in this group of drugs does not stem from this, but from work undertaken by Fleckenstein in the mid 1960's. Fleckenstein was using electro-physiological techniques to investigate the properties of two newly synthesized compounds, prenylamine and verapamil. Fleckenstein and colleagues found that these drugs were able to uncouple excitation-contraction coupling in the heart, and that this could be reversed by the addition of Ca^{++}. He later found that they produced a selective inhibition of the slow channel component of cardiac action potentials similar to that of Mn^{++}. It was decided that these properties were distinct enough to warrant the formation of a new class of drugs, the "calcium antagonists" (Fleckenstein *et al* 1969). By early in the 1970's the dihydropyridines had been discovered and were being developed (Vater *et al* 1972). By the mid 70's work was increasing; new drugs, including nitrendipine, were discovered and their pharmacology and potential uses are currently being widely investigated.

3.1.2 Structure of nitrendipine.

Calcium antagonists are a very heterogeneous group of compounds. Nitrendipine is a dialkyl4-aryl-1,4-dihydropyridine-3,5-di-carboxylate (dihydropyridine). The dihydropyridines are devoid of any basic activity due to the resonance of the lone pair electron on the nitrogen atom with the carbonyl groups (Meyer *et al* 1983). The structure of nitrendipine is shown below.



One important characteristic of nitrendipine, distinct from some other dihydropyridines is the presence of non-identical ester groups. This has been shown in the examination of structure activity relationships to account for the higher vasodilatory, or antihypertensive, activity compared with symmetrically substituted dihydropyridines (Meyer 1984).

3.1.3 Pharmacodynamics of nitrendipine.

The electrophysiological activity of calcium entry blockers (also known as calcium antagonists & slow channel blockers) in cardiac tissue has been thoroughly investigated (Cohen *et al* 1984, Katz 1986, Nayler 1980, Nayler *et al* 1984, 1986, 1988), but less is known about their electrophysiological effects in smooth muscle.

Two types of channels (slow & fast) can be identified in the heart and vasculature, based on differences in their action potential. The rapid initiation of the action potential characteristic of fast channels is mediated by the movement of sodium ions. Calcium crosses the cell membrane through voltage-dependent, Ca^{++} selective, "slow" channels and so initiates the "slow" response and provokes excitation-contraction coupling. In vascular smooth muscle, Ca^{++} ions enter the cell through "receptor-operated" channels and mediate the excitation-contraction coupling (Hofmann 1985, Godfraind *et al* 1986). Rogart *et al* (1985) have proposed that there are "high affinity" binding sites related to "slow" channels in vascular smooth muscle and "low affinity" binding sites associated with cardiac muscle. Recent research using rat mesentery has suggested that the slow channel in vascular smooth muscle is more sensitive to nitrendipine blockade than the slow channels in cardiac muscle (Bean *et al* 1986, Triggle 1986, Ross & Monis 1988). This suggests that the slow channels in vascular smooth muscle may be different from those in cardiac muscle (Fleckenstein 1977, Van Breemen *et al* 1980). In clinical trials in patients with coronary heart disease, angina pectoris and borderline hypertension, nitrendipine did not alter conduction through sinus or atrioventricular nodes (Van Zwieten & Timmermans 1983, Rutsch & Schmutzler 1984). Similarly nitrendipine given to patients with essential hypertension had no

significant effect on resting PR, QRS, QT intervals or on AV conduction (Maltz *et al* 1986).

Total peripheral resistance has been shown to be reduced in both normotensive and spontaneously hypertensive rats following a single dose of nitrendipine (Pegram *et al* 1984). The reduction in peripheral resistance is greater in spontaneously hypertensive rats after chronic treatment; however, there is evidence that it is reduced or absent following chronic treatment in normotensive animals (Pegram *et al* 1984, Dunn *et al* 1984). Single doses of nitrendipine have also been shown to produce a reduction in mean, systolic and diastolic blood pressure via a reduction in total peripheral resistance in hypertensive patients. These levels remain depressed from 8-24 hours after the dose (Andren *et al* 1982, Burris *et al* 1982, Hanson *et al* 1983). Acute dosing with nitrendipine in normotensive subjects also produced similar effects, but somewhat less pronounced (Pedersen 1983, Wallia *et al* 1985). The antihypertensive effect of nitrendipine is sustained during long term administration in hypertensive patients (Nannan *et al* 1984, Fouad *et al* 1982, 1984, Pedrinelli *et al* 1986). The reflex increase in heart rate following acute oral administration of nitrendipine in both hypertensive patients and normal volunteers is around 6-32% (Nannan *et al* 1984, Debbas *et al* 1984, Frohlich 1985). This increase is reduced or absent following long term treatment in man (Fouad *et al* 1982, 1984, Nannan *et al* 1984). The reflex tachycardia associated with a decrease in total peripheral resistance following nitrendipine administration is observed in normotensive but not hypertensive rats (Pegram *et al* 1984). Nitrendipine exhibited a similar hypotensive action during exercise (Nannan *et al* 1984) although the reflex increase in heart rate that accompanied the reduction in blood pressure was less pronounced. Overall the evidence suggests that nitrendipine reduces

total peripheral resistance preferentially in the presence of an abnormally raised tone.

In normotensive and spontaneously hypertensive rats, acute and chronic oral nitrendipine treatment decreased renal vascular resistance (Dunn *et al* 1984, Pegram *et al* 1984). The effects of nitrendipine on renal haemodynamics in human subjects has been shown to be minimal following both acute and chronic administration (Fouad *et al* 1982, 1984, Wallia *et al* 1985). Chronic nitrendipine treatment has been shown to increase plasma renin activity (Fouad *et al* 1982) although there is some controversy over this effect. Increases that were observed have been shown to decline over time and be normalised at the end of 12 months therapy (Weber & Drayer 1984, Leonetti & Zanchetti 1985). Further it has been suggested that this putative effect is not related to the antihypertensive effect of nitrendipine, as the magnitude of hypotensive effect is positively correlated with low initial plasma renin activity (Kiowski *et al* 1985, McMahon 1986). Acute administration of nitrendipine has been shown to produce modest natriuresis and diuresis without increased potassium loss (Wallia *et al* 1985, Hall & Hungerford 1984). The evidence of these actions continuing over the long term, however, is less conclusive. Since nitrendipine does not appear to have a large effect on renal blood flow or glomerular filtration rate its acute natriuretic and diuretic effects may be mediated by other than haemodynamic changes. Wallia (1985) postulates that nitrendipine may block the reabsorption of sodium by a direct action on the proximal tubule of the kidney.

The location of the ^{3}H-nitrendipine binding is separate from α_1-adrenoceptors (Ross & Monis 1988, Triggle 1986, Dompert & Traber 1984, Nayler *et al* 1984, Motulsky *et al* 1983). Studies *in vivo* have shown that nitrendipine blocks pressor responses to both exogenous and endogenous

noradrenaline (Pedrinelli & Tarazi 1984), and to vasopressin and angiotensin II (Pedrinelli & Tarazi 1985a). While other work has suggested that nitrendipine reduces pressor responses to noradrenaline but not to angiotensin II (Simon & Snyder 1984, Lefevre-Borg *et al* 1988), the exact involvement of Ca^{++}-blockers and the sympathetic nervous system is contentious. There is evidence that nitrendipine both does and does not alter transmitter release. The results vary between species, vascular beds and methods used. In general it seems that concentrations of nitrendipine that are required to produce alterations in neurotransmitter release are considerably higher than those required to effect the response to noradrenaline. It is questionable whether the concentrations required to alter transmitter release have any therapeutic relevance. The only effect nitrendipine has on sympathetic transmission at doses achieved during clinical usage appear to be via postjunctional effects. These can vary between vascular beds depending on the relative populations of postjunctional α_1- and α_2-adrenoceptors. This is because noradrenaline is more potent at α_1-receptors while nitrendipine appears to be more potent at α_2-receptors, although increasing evidence suggests that the difference in Ca^{++}-antagonist potency at α-adrenoceptors may be due to variation in receptor reserve (Pedrinelli & Tarazi 1985a, 1985b). For a more detailed discussion of the effects of Ca^{++}-antagonists on sympathetic neuroeffector function the reader is directed to a review of the subject by Eikenburg and Lokhandwala (1986).

When given in therapeutic doses, nitrendipine does not alter serum glucose or lipid concentrations, although it was shown to inhibit glucose transport in adipocytes (Christensen & McDonald 1985). Similarly in chronic studies nitrendipine was shown to have no effects on cholesterol, total triglycerides or high density lipoproteins (Ferrara *et al* 1985).

In some animal models nitrendipine has been shown to have other effects; there is evidence that it dilates cerebral blood vessels and reduces pulmonary artery vasoconstriction with a subsequent decrease in pressure. A single clinical study in patients with pulmonary hypertension demonstrated similar effects. In addition to its ability to relax vascular smooth muscle, nitrendipine also inhibits tracheobronchial and uterine contractions in animals. The clinical implications of these effects are, however, uncertain (Goa & Sorkin 1987). Interestingly nitrendipine has been shown to decrease free cellular Ca^{++} in platelets and this decrease is highly correlated with its hypotensive action. It has been suggested that the decrease in platelet Ca^{++} may reflect a decrease in the overall free Ca^{++} status of patients, which has been shown to be raised in hypertension (Erne *et al* 1984).

3.1.4 Selectivity of nitrendipine.

Calcium channels are present in a wide variety of tissues including the heart and smooth muscle, and Ca^{++} flux is vital to many physiological processes (Godfraind *et al* 1986). ^{3}H-nitrendipine binding sites have been demonstrated to be associated with Ca^{++} channels in a wide variety of tissues including vascular and other smooth muscle, brain and heart. These binding sites are generally considered to be of a single class which reversibly bind dihydropyridines with a high affinity (K_d 0·1-1·0 nm); binding sites present in skeletal muscle are, however different with a much lower affinity (K_d 5nm) (Nayler *et al* 1984, Dompert & Traber 1984, Godfraind *et al* 1986, Ross & Monis 1988). Despite the variety of potential binding sites nitrendipine has been shown to exhibit a very high degree of

vasoselectivity (Taira 1987). The explanation of this selectivity is unclear, but Bean (1983) has suggested the following theory:- Calcium channels can exist in one of three states; resting (closed), activated (open) and inactivated (closed) (Tsein 1983, Triggle 1986). The channels are able to cycle between these three states. When a cell is polarised, these channels exist dominantly, or exclusively in a resting state. The channels are voltage operated, that is their state is dependent on the membrane potential. When the cell is depolarised they shift to the open state; if they remain depolarised at a high enough level for long enough they change to the inactivated state. Nitrendipine has the option of binding to any of these states but its affinity for the inactivated state is a thousand times higher than for the other states (Taira 1987). On binding to the channel nitrendipine stabilises it in that configuration and thus prevents the entry of Ca^{++}.

In working cardiac muscle cells the channels become inactivated during the plateau of the action potential. However the time the channels remain in this state (<1 s) is much less than the time required for nitrendipine binding. Even if the nitrendipine does bind it dissociates promptly during electrical diastole. Thus, it is "difficult" for nitrendipine to bind to working cardiac muscle. In contrast, in vascular smooth muscle the membrane potential is less than the diastolic membrane potential in cardiac cells. The vascular muscle is partially depolarised all the time, therefore the Ca^{++}-channels are more likely to be in the inactivated state, and to remain there for long enough to allow nitrendipine to bind. This same principle may explain the "finer" specificity such as the preference for particular vascular beds, but there are, however, questions still to be answered. Why are other Ca^{++}-antagonists (eg verapamil) which are able to bind to the Ca^{++}-channel in its inactivated state (McDonald *et al* 1980) not more vasoactive? Why does nitrendipine exhibit inactivity or low activity

in neural systems when binding sites are present (Triggle & Janis 1984, Spedding & Middlemasm 1985)? These inconsistencies may be explained by differences in the relationship between the ^{3}H-nitrendipine binding site and the Ca^{++}-channel, or possibly by the relative importance of "activator" Ca^{++} from internal sources. More work is required to explain fully the basis of Ca^{++}-antagonist selectivity.

3.1.5 Pharmacokinetics and metabolism of nitrendipine.

Investigation of the absorption of nitrendipine in man has shown that about 80% of an oral 20mg dose was absorbed in healthy volunteers, and peak plasma concentrations of between 4·7 and 41·6 µg/L were achieved within 1 to 2 hours. Linear increases in area under the plasma concentration-time curves were observed after oral doses of up to 40mg/kg indicating that liver enzyme saturation had not occurred (Johnson *et al* 1986, Hanson *et al* 1983). Studies have shown that nitrendipine has a relatively low and variable bioavailability of 16 ±6%, and that the bioavailability of tablets relative to solution was 75% (Kann *et al* 1984) A study in healthy volunteers has shown that following a single i.v. nitrendipine dose of 2mg the volume of distribution of the central compartment obtained at steady state (VD_{ss}) was 2·04 L/kg. The study also showed that nitrendipine is about 98% bound to plasma proteins (Raemsch & Sommer 1984).

Nitrendipine is very extensively metabolised in the liver to one of five major metabolites, each one of them around a thousand times less pharmacologically potent than nitrendipine. The major routes of metabolism are dehydrogenation to the pyridine analogue, cleavage of the ester groups by hydrolysis to carboxylic acids and hydroxylation of the methyl groups

with subsequent glucuronide conjugation in the bile. About 45% of a dose of nitrendipine is excreted in the urine in the form of inactive metabolites during the first 24 hours. Around 8% is excreted in a similar form in the faeces and the rest is eliminated in the bile. Less than 0·1% of an oral dose appears as unchanged drug in the urine. This high first pass effect explains the relatively low bioavailability of the drug (Raemsch & Sommer 1984, Kann *et al* 1984). In healthy volunteers and hypertensive patients the half life of nitrendipine varied from 2 to 24 hours, with a mean value of about 12 hours (Raemsch & Sommer 1984, Kann *et al* 1984, Hanson *et al* 1983).

The hypotensive effect of nitrendipine is evident at concentrations that are undetectable in the plasma. It has been postulated that the antihypertensive effect of nitrendipine may correlate with its activity at the calcium channel rather than with its plasma concentration (Aronoff 1984).

It should be noted that some of the variation in the pharmacokinetics of nitrendipine may be due to the methods of detection employed. In earlier studies high performance liquid chromatography (HPLC) and radiolabelled receptor methods were used to measure plasma and urine nitrendipine concentrations. In more recent studies mass spectrometric or capillary gas chromatography with electron capture were used. These later methods are both more specific and more sensitive.

3.1.6 Toxicology of nitrendipine.

Results from acute toxicity studies have shown that nitrendipine is only weakly toxic when given orally with an LD_{50} value of greater than 10000 mg/kg in the rat. When administered by an intravenous route it was, however, considerably more toxic with an LD_{50} value of 12·6 mg/kg in rats. The major symptoms of toxicity were central and included reduced motility, cyanosis, vomiting and convulsions. The intensity and time course of these symptoms depended on the route of nitrendipine administration. After oral administration symptoms persisted for between 1 and 8 days and were followed by death; however, after i.v. administration death was immediate.

Chronic toxicity studies with doses of nitrendipine of up to 30 mg/kg/day for 2 years produced no signs of toxic effects in rats. At doses of 147 mg/kg/day the growth rate of rats was retarded and signs of liver damage were apparent; some individuals also showed signs of myocardial scarring which was thought to be due to hypoxic damage that occurred as a result of the initial high degree of hypotension and tachycardia. The chronic investigations also showed that nitrendipine possessed no apparent adverse effects on the fertility of rats and no teratogenic or mutagenic effects (Hoffmann 1984).

3.1.7 Mechanisms of the antihypertensive action of nitrendipine.

The primary action of nitrendipine is to inhibit the movement of Ca^{++} through the voltage-dependent "slow" channels in plasma membranes (see sections 3.1.3 & 3.1.4). Elevated levels of free Ca^{++} in the cytosol are

known to enhance vascular tone (Rosendorff 1984), and it has been suggested that levels of Ca^{++} in the vascular smooth muscle are raised in hypertension (Aoki *et al* 1982, Erne *et al* 1984). The main antihypertensive action of nitrendipine is a result of its blockade of Ca^{++}-dependent contractile activity in vascular smooth muscle. This results in a relaxation of peripheral vascular tone, a subsequent decrease in systemic vascular resistance, resulting in a reduction of blood pressure. In addition to this main effect, nitrendipine possesses some other properties which may contribute to its hypotensive mode of action.

The natriuretic effect of nitrendipine (section 3.1.3) seems to play a part, in that it relieves sodium build up that is often present in hypertension and so reduces water retention. The reduction in volume load this causes may play a significant part in its antihypertensive effect (Garthoff *et al* 1983, Hall & Hungerford 1984). Nitrendipine has also been shown to normalise renal blood flow and glomerular filtration rate in angiotensin II induced hypertension in rats (Sterzel *et al* 1984).

Chronic nitrendipine treatment has been shown to reduce generalized vasculopathy and cardiac hypertrophy and these effects may also contribute to maintaining its antihypertensive action. There is also evidence that nitrendipine treatment preserves tissue integrity and has been shown to increase life span in malignant hypertension and in stroke-prone hypertensive rats. It has been postulated that these effects are a result of its prevention of generalised deleterious calcium overload (Kazda *et al* 1984, Kazda 1986).

3.2 METHODS.

3.2.1 Investigation of blood pressure in the conscious rat.

The initial study of blood pressure was undertaken with normotensive male Wistar rats (University of Bath strain) weighing approximately 200g. Systolic blood pressure was measured using a non-invasive "tail-cuff" technique. The methodological details of the procedure are described in section 2.2.1.

Blood pressure was measured before and two hours after the daily dose of either nitrendipine or polyethylene glycol (PEG). During the first week of dosing all animals were dosed with 5% PEG alone; in order that they became accustomed to the procedures involved. In the subsequent three weeks the treated group received 3mg/Kg nitrendipine in a 5% PEG vehicle, while the control group received the 5% PEG vehicle alone. In both cases drugs were orally administered (1ml/100g) daily, blood pressure was not, however, measured over weekend periods.

This work was repeated with spontaneously hypertensive Japanese Okamoto rats (University of Bath strain) weighing approximately 200g.

The investigation of the effects of nitrendipine on blood pressure in both conscious normotensive and hypertensive rats was undertaken in parallel with the study investigating the effects of atenolol (section 2.2.1).

3.2.2 Investigation of heart rate in the conscious rat.

Heart rate was calculated from the pulse pressure waves recorded during the measurement of blood pressure. This was undertaken with both normotensive Wistar and spontaneously hypertensive Japanese Okamoto rats.

The details of how this was calculated is given in section 2.2.2; the effects of nitrendipine on heart rate was was examined in the same study as the examination of the effects of atenolol.

3.2.3 Assessment of isoprenaline challenge.

β-adrenoceptor blockade was assessed using an anaesthetised rat preparation to investigate the dose response response curve to bolus i.v. injections of isoprenaline.

The protocol, described in detail in section 2.2.3, was used to assess β-adrenoceptor blockade following 7 and 21 days pretreatment with either nitrendipine (3mg/kg p.o. in 5% PEG, 1ml/100g) or 5% PEG (1ml/100g). The procedure was followed 24 hours after the last dose of either nitrendipine or PEG.

3.2.4 The *in-situ* blood perfused mesentery method.

The effect of chronic calcium channel antagonism by nitrendipine on adrenergic neurotransmission was investigated using the *in-situ* blood perfused mesentery method of Jackson and Campbell (1980a). Details of the method used is given in section 2.2.4., the method is shown diagrammatically in figure 3.

The protocol was used to investigate mesenteric responses to exogenous noradrenaline (20-2000ng in 0·9% w/v NaCl) and periarterial electrical stimulation (15v rectangular pulses of 1ms duration for 20s, 2-35Hz). This work was undertaken 24 hours after the last dose of either nitrendipine (3mg/kg in 5%, 1ml/100g) or 5% PEG (1ml/100g) after both 7 and 21 days pretreatment. This work was carried out with both normotensive male Wistar and spontaneously hypertensive Japanese Okamoto rats (University of Bath strain, 300-330g).

3.2.5 General considerations.

3.2.5(i) Animal husbandry.

Normotensive Wistar and spontaneously hypertensive Japanese Okamoto rats were used in the investigation of the chronic effects of nitrendipine. These rats were housed at the University of Bath with a 12 hour light/dark

cycle at a temperature of 20-22°C and a relative humidity of 40-60%. The animals were provided with 'Labsure' CRM diet and tap water *ad libitum*, and were housed in groups.

Details of animal husbandry are described in greater detail in section 2.2.7(i) on page 59.

3.2.5(ii) Statistical analysis.

The blood pressure and heart rate data recorded from conscious animals was analysed using the values obtained before daily dosing. The normality of the data was tested with the Kolmogorov-Smirnov procedure and was checked by plotting a frequency histogram with a normal curve superimposed. Analysis of variance (ANOVA) with Fisher's LSD, Tukey's HSD and Scheffe's test follow-ups were performed on the week I data to discover any differences between groups before "active" treatment. The ANOVA and follow-ups were repeated with the week IV data to examine the differences following chronic treatment with nitrendipine. The ANOVA's examined not only the differences between animals treated with nitrendipine and the controls but differences between the other treatment groups as well. Unpaired Student's t-tests or Mann-Whitney U tests were used to compare two sample means depending on whether the data was normal or non-parametric.

In all experiments animals were randomly assigned to groups and treatments randomly allotted to these groups. The statistical analysis was carried out by computer using the SPSS-X package. Full details are given in section 2.2.7(iii) on page 61.

3.2.5(iii) Drugs and general chemicals.

In addition to the list of drugs and chemicals given in section 2.2.7(iv) on page 63 the 1,4-dihydropyridine nitrendipine was used. This was kindly provided by Bayer Pharmaceuticals PLC.

The light sensitivity of nitrendipine dictated that great care was taken to avoid its exposure to light, both in its storage in solid form and during the daily preparation of solutions. Nitrendipine was solubilised in polyethylene glycol (PEG) at 60°C, which was then diluted to a 5% solution with deionised water.

3.3 RESULTS.

3.3.1 Results of the investigation of blood pressure.

3.3.1(i) Results from conscious normotensive Wistar rats.

Throughout the control period (week I), when all animals were dosed with 5% polyethylene glycol (PEG) alone, there was no significant difference between the two groups of animals. In both groups blood pressure was elevated on the first day of the procedure; this then normalised at around 151 mmHg.

Both groups had a mean blood pressure of around 154 (max SE 2·9) mmHg at the start of "active" treatment in week II. During the first week of dosing with nitrendipine the blood pressure of the treated group fell to 132 (±4·2) mmHg while that of the control group remained around 153 (±1·5) mmHg. The diurnal variation in blood pressure (pre and post dosing) followed a similar pattern to that described for the atenolol treated animals. The variation was initially large and diminished over the dosing period. The attenuation of blood pressure observed; was maintained throughout the subsequent period of nitrendipine treatment. The blood pressure of the control group remained fairly constant throughout this treatment period with the group having a mean systolic blood pressure of 153 (±1·6) mmHg during the final week of dosing. The animals treated with nitrendipine had a mean systolic blood pressure of 143 (±2·1) mmHg during the same period. This difference was found to be statistically highly significantly different ($p<0·0001$) using an ANOVA procedure. Fisher's LSD follow-up to ANOVA indicated that there was a significant ($p<0·05$)

difference between the group treated with nitrendipine and the group treated with atenolol. That is animals treated with nitrendipine had a higher blood pressure during week IV than animals treated with atenolol; although it was still reduced from the level of the control group. This difference was not apparent when the data was examined using either the Tukey HSD or Scheffe procedure follow-ups. The graph of these results (figure 30, page 131) shows that the blood pressure of the nitrendipine group follows a similar pattern to that described for animals treated with atenolol (figure 10, page 75). The reduction of blood pressure is, however, slower in onset and less pronounced; although there is no clear statistical evidence to support this.

3.3.1(ii) Results from conscious spontaneously hypertensive rats.

During the first week (control) of the investigation there was no significant difference between the two groups. As previously described (section 2.3.1(ii)) the systolic blood pressure of the hypertensive rats was found to be around 50 mmHg higher than their normotensive counterparts during control PEG treatment.

On the initiation of "active" treatment with nitrendipine the blood pressure of the treated animals was reduced compared with that of the PEG treated control animals. This reduction occurred during the first week of nitrendipine treatment and was maintained throughout the dosing period. The blood pressure of the spontaneously hypertensive animals was at no time reduced to normotensive levels. The reduction was, however, found to be highly statistically significant ($p<0{\cdot}0001$) using an ANOVA. Mean systolic blood pressure of the treated group had been reduced to 196 ($\pm 2{\cdot}7$) mmHg

from the control level of 226 (±0·6) mmHg during the final week of dosing. Unlike the situation described for the normotensive groups in the previous section there was no evidence of any difference between reductions observed with nitrendipine and atenolol. The daily variation in blood pressure measured before and after dosing was as previously described with the difference becoming smaller over time.

The graph of conscious systolic blood pressure over the treatment period for control and nitrendipine treated hypertensives is shown in figure 31 on page 132.

3.3.2 Results of the investigation of heart rate.

3.3.2(i) Results from conscious normotensive rats.

There was found to be no significant difference between treatment groups during week I when all animals received PEG alone. In the initial part of the first week heart rate was around 460 b/min; this decreased over the control week.

During the "active" dosing period (weeks II-IV) the heart rate of the control group varied around 400 b/min. In general heart rate in the animals treated with nitrendipine was higher than that recorded in control animals; there was, however, some variation. Data from the last week of dosing was analysed and the nitrendipine treated group was found to have a significantly ($p<0·05$) raised heart rate compared with that of the control group. The nitrendipine treated animals also had significantly ($p<0·05$)

increased heart rates compared with those measured in atenolol treated animals. These results are shown as a graph in figure 32 on page 133.

3.3.2(ii) Results from conscious spontaneously hypertensive rats.

The results of the changes in heart rate measured in hypertensive animals is similar to that described in normotensives in that there is an overall tendency for heart rate to decrease over the experimental period irrespective of treatment. Also, there was no significant difference between the groups before "active" treatment commenced. However, Nitrendipine treatment did not produce any significant difference in heart rate between the treated and control groups in the hypertensive animals. A significant ($p<0{\cdot}05$) difference between hypertensive animals treated with nitrendipine and those treated with atenolol was, however, observed.

Figure 33 on page 134 is a graph of heart rate over the experimental period.

3.3.3 Results of the assessment of isoprenaline challenge.

Pretreatment with nitrendipine for 7 days resulted in an increase in response to low doses of isoprenaline. The lower portion of the dose response curve measured after 7 days nitrendipine was shifted up the x-axis compared to the control curve. This shift in response was found to be

statistically significant ($p<0.05$) at isoprenaline doses up to 0·02ng. This is clearly illustrated in the graph shown in figure 34.

A similar pattern of changes to the isoprenaline dose response curves was apparent following 21 days nitrendipine treatment. Again the responses observed to doses of isoprenaline at doses up to 0·02ng were raised compared to control responses. This increase was found to be statistically significant (at between $p<0.05$ and $p<0.01$). The increase in response to isoprenaline did not result in a parallel shift in the dose response curves after 7 or 21 days pretreatment.

3.3.4 Results from the *in-situ* blood perfused mesentery model.

3.3.4(i) Results from normotensive Wistar rats.

The noradrenaline dose response curves obtained after 7 days pretreatment with either PEG or nitrendipine are shown in figure 36. Nitrendipine pretreatment did not produce any significant changes in the dose response curve to exogenous noradrenaline compared with the PEG treated control group.

Figure 37 shows the frequency response curve to periarterial electrical stimulation following 7 days nitrendipine pretreatment. The curve obtained from the nitrendipine pretreated animals shows that there is

no evidence of any significant change in response to periarterial electrical stimulation.

The dose response curves to exogenous noradrenaline obtained after 21 days pretreatment with either PEG or nitrendipine are shown in figure 38. The response curve from the nitrendipine treated animals is almost superimposed on the curve obtained from the control animals. There is no statistically significant difference between the two groups.

The mesenteric response to periarterial electrical stimulation following 21 days pretreatment is shown in figure 39. The responses obtained from animals pretreated with nitrendipine are reduced in comparison with control responses. This attenuation is significant, with a $p<0{\cdot}01$ at frequencies up to 8Hz and with a $p<0{\cdot}05$ at higher frequencies. The two response curves are of a similar shape and are parallel.

The mean systemic blood pressure, mean mesenteric blood pressure and mesenteric resistance measured in the anaesthetised animals before responses to noradrenaline and electrical stimulation were ascertained are shown in appendix 2. There was no over-all differences in any of these parameters between animals pretreated with PEG or nitrendipine. There was a slight reduction in mean systemic and mesenteric blood pressures following 7 days pretreatment, this was no longer apparent after 21 days.

3.3.2(ii) Results obtained from hypertensive rats.

The noradrenaline dose response curves and the stimulation response curves obtained in spontaneously hypertensive animals following 7 and 21 days pretreatment with PEG (control) are similar to the control curves obtained from normotensive animals. There is, however, a greater response to both exogenous noradrenaline and periarterial electrical stimulation in the hypertensive animals.

The dose response curve to noradrenaline following 7 days pretreatment with either nitrendipine or PEG is shown in figure 40. The graph shows that following 7 days nitrendipine pretreatment there is a reduced response to noradrenalin resulting in a parallel shift of the dose response curve. This attenuation in noradrenaline response was found to be statistically significant ($p<0{\cdot}05$ - $p<0{\cdot}01$).

Figure 41 shows the stimulation response curves following 7 days pretreatment in spontaneously hypertensive animals. This graph also shows a reduced response; in this case a significant ($p<0{\cdot}01$) attenuation of mesenteric response to periarterial electrical stimulation.

The dose response curves to exogenous noradrenaline after 21 days pretreatment is shown in figure 42. There is a reduction in mesenteric response to all doses of exogenous noradrenaline. This reduction was found to be statistically significantly different ($p<0{\cdot}01$).

The mesenteric response to periarterial electrical stimulation was also reduced following 21 days pretreatment with nitrendipine. This reduction was statistically significant ($p<0{\cdot}05$) at all frequencies examined greater than 2Hz. Figure 43 shows the stimulation response curves obtained after 21 days pretreatment with either PEG or nitrendipine.

The stimulation response and noradrenaline dose-response curves following 21 days nitrendipine pretreatment both show a reduction compared to control curves. This parallel shift in the curves is similar to that seen in spontaneously hypertensive animals after 7 days nitrendipine pretreatment. Although there is some evidence to suggest that the attenuation is greater following 21 days pretreatment.

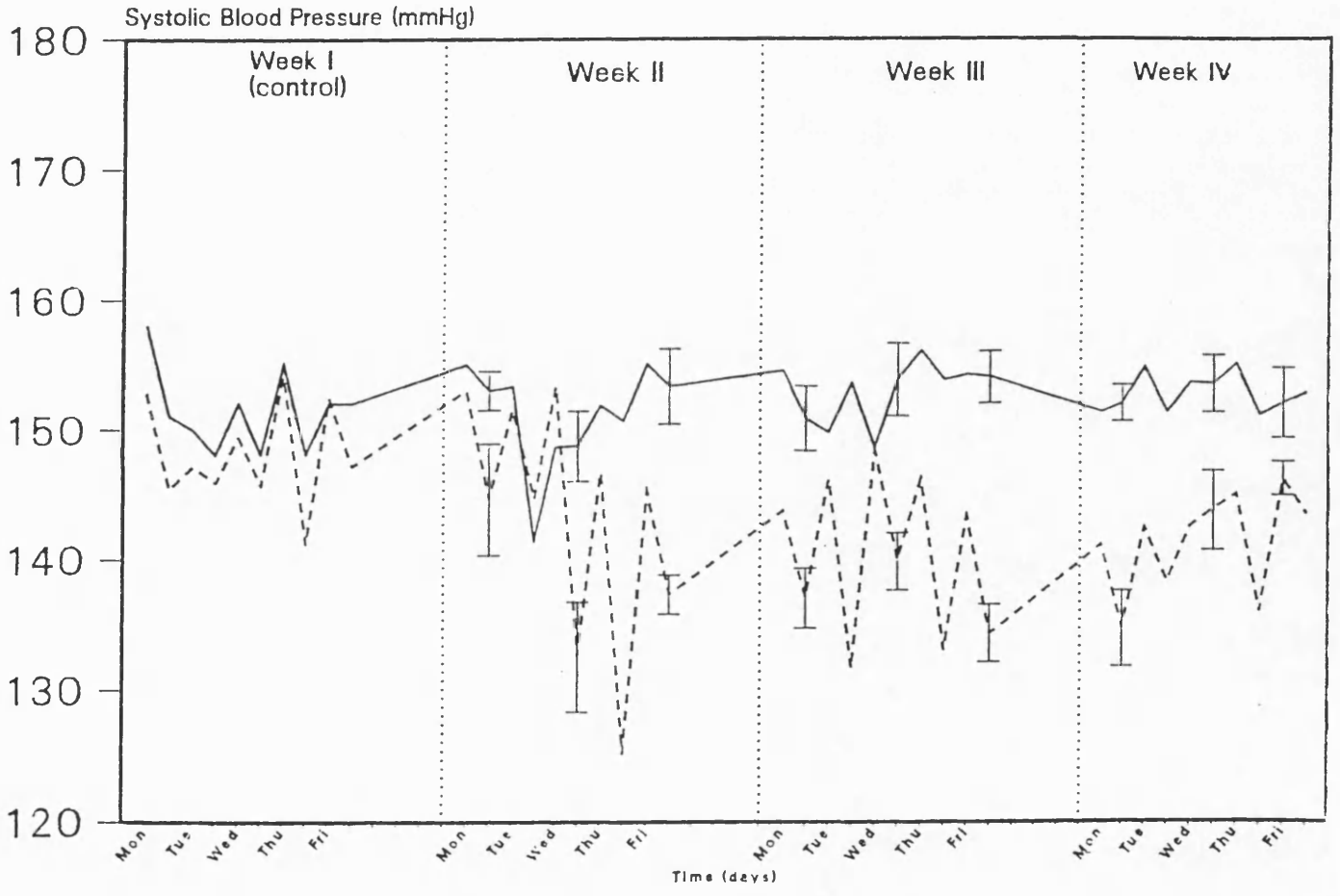
The data in appendix 2 shows that there was a tendency for an increase in "resting": mean systemic blood pressure, mean mesenteric blood pressure and mesenteric resistance following 7 days nitrendipine pretreatment in the anaesthetised rat. These parameters are, however, all reduced after 21 days nitrendipine pretreatment.

In the following graphs (figures 30-43), statistical difference was calculated between the values from control animals (treated with PEG) and the corresponding values from nitrendipine treated animals. This difference is shown by the following notation :

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE 30

Graph showing the effect of nitrendipine (3mg/kg) p.o. on systolic blood pressure in the conscious normotensive male Wistar rat.

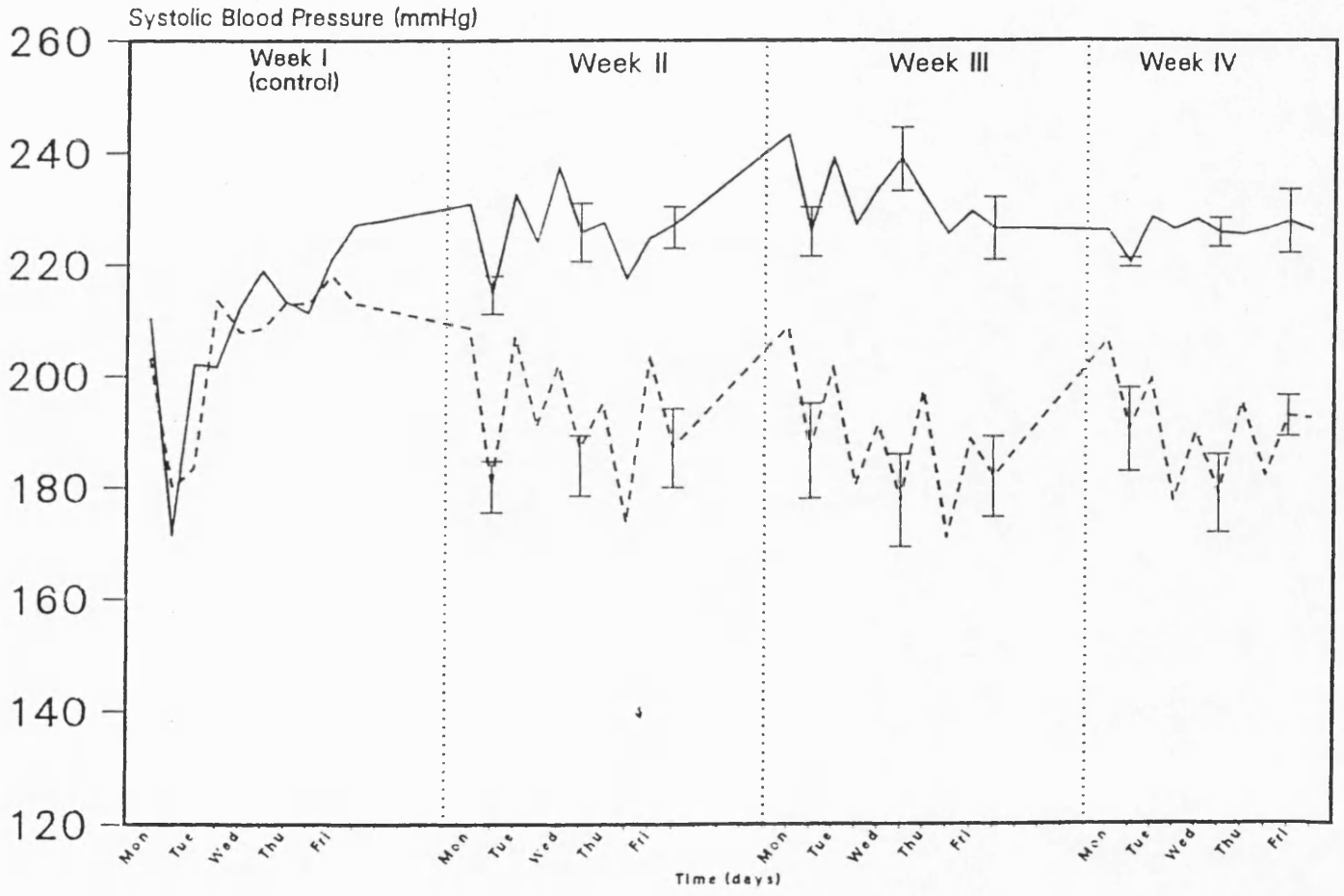


----- Nitrendipine (3mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=8.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=6.

FIGURE 31

Graph showing the effect of nitrendipine (3mg/kg) p.o. on systolic blood pressure in the conscious spontaneously hypertensive male Japanese Okamoto rat.

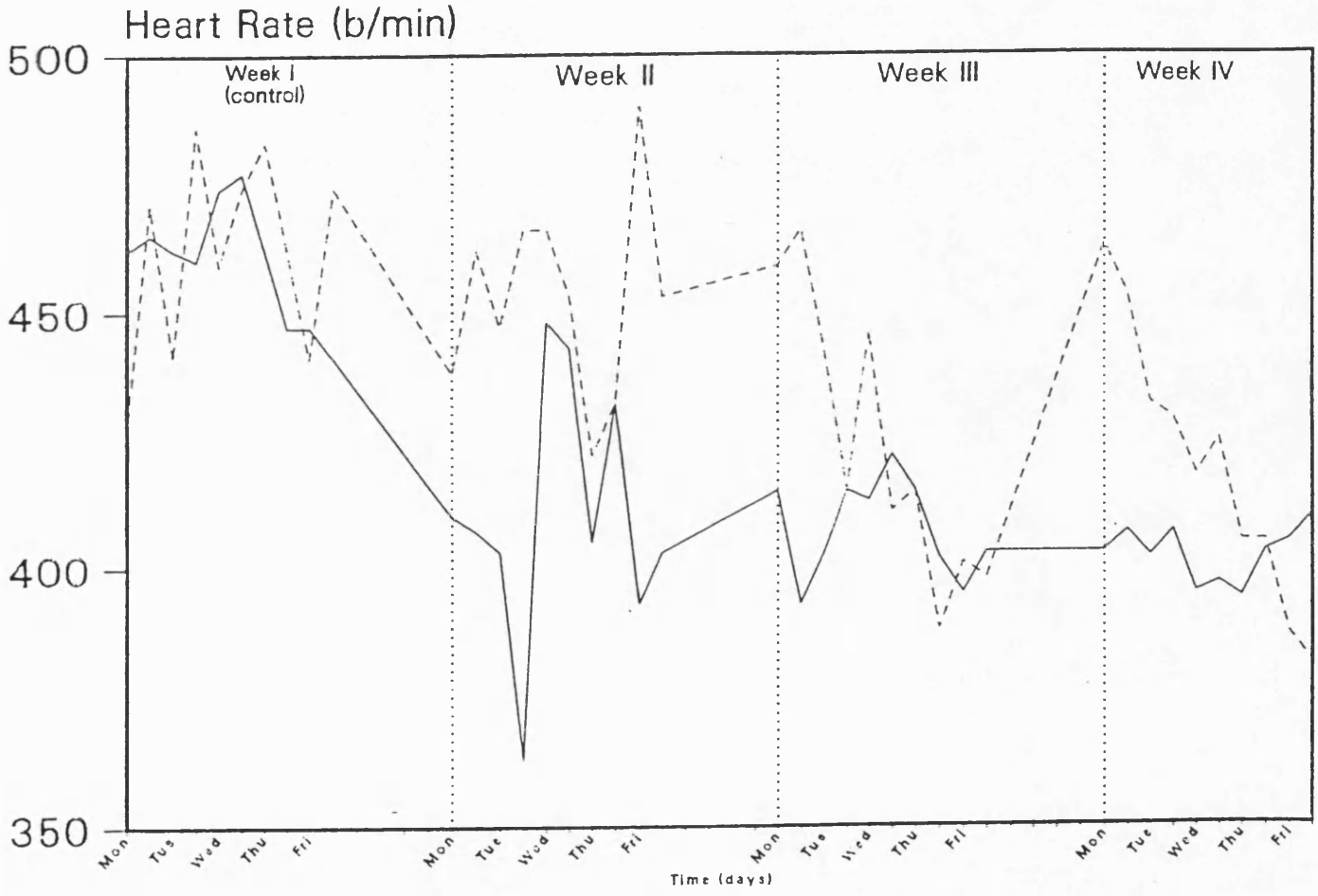


----- Nitrendipine (3mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=4.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=4.

FIGURE 32

Graph showing the effect of nitrendipine (3mg/kg) p.o. on heart rate in the conscious normotensive male Wistar rat.

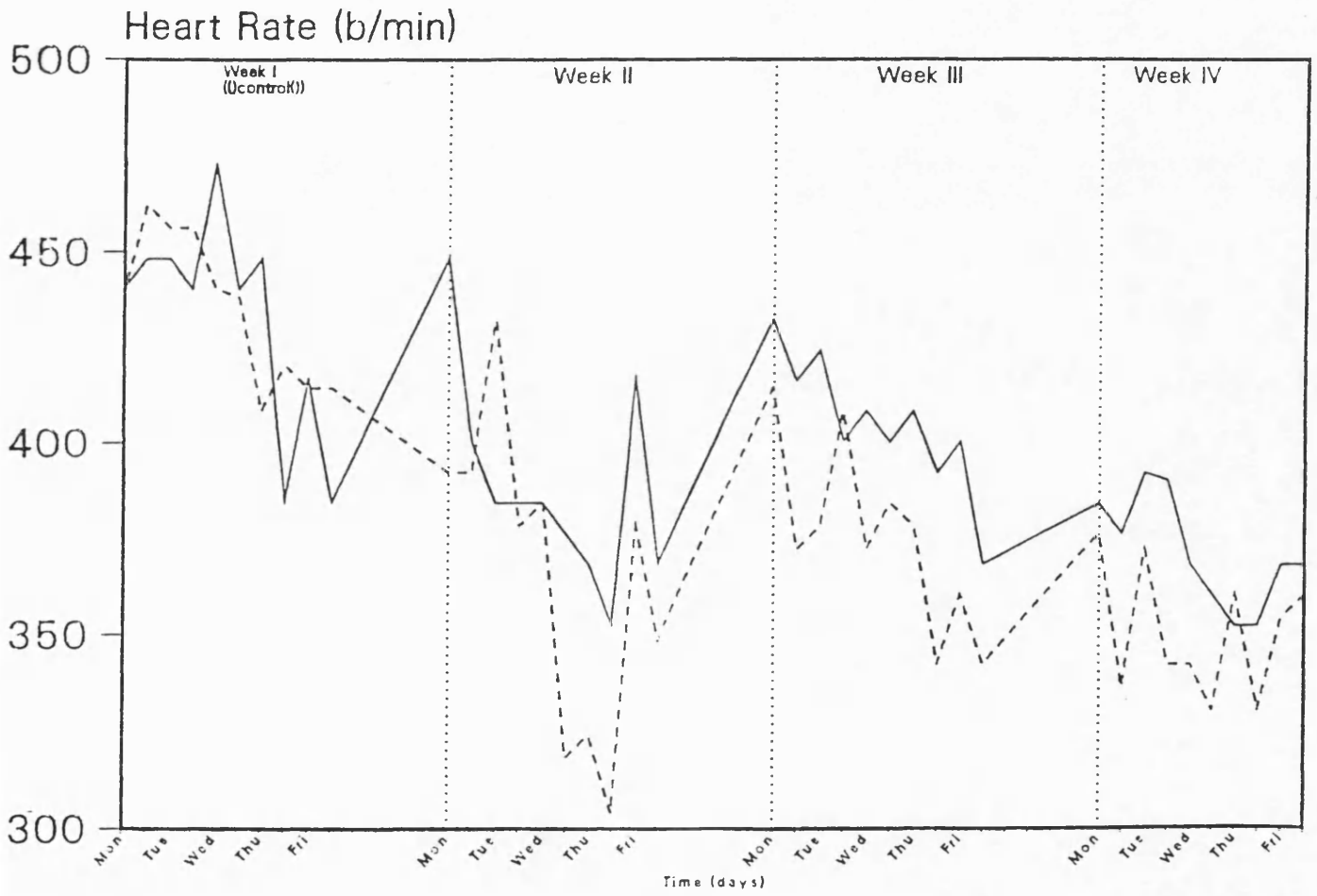


----- Nitrendipine (3mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=8.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=6.

FIGURE 33

Graph showing the effect of nitrendipine (3mg/kg) p.o. on heart rate in the conscious spontaneously hypertensive male Japanese Okamoto rat.

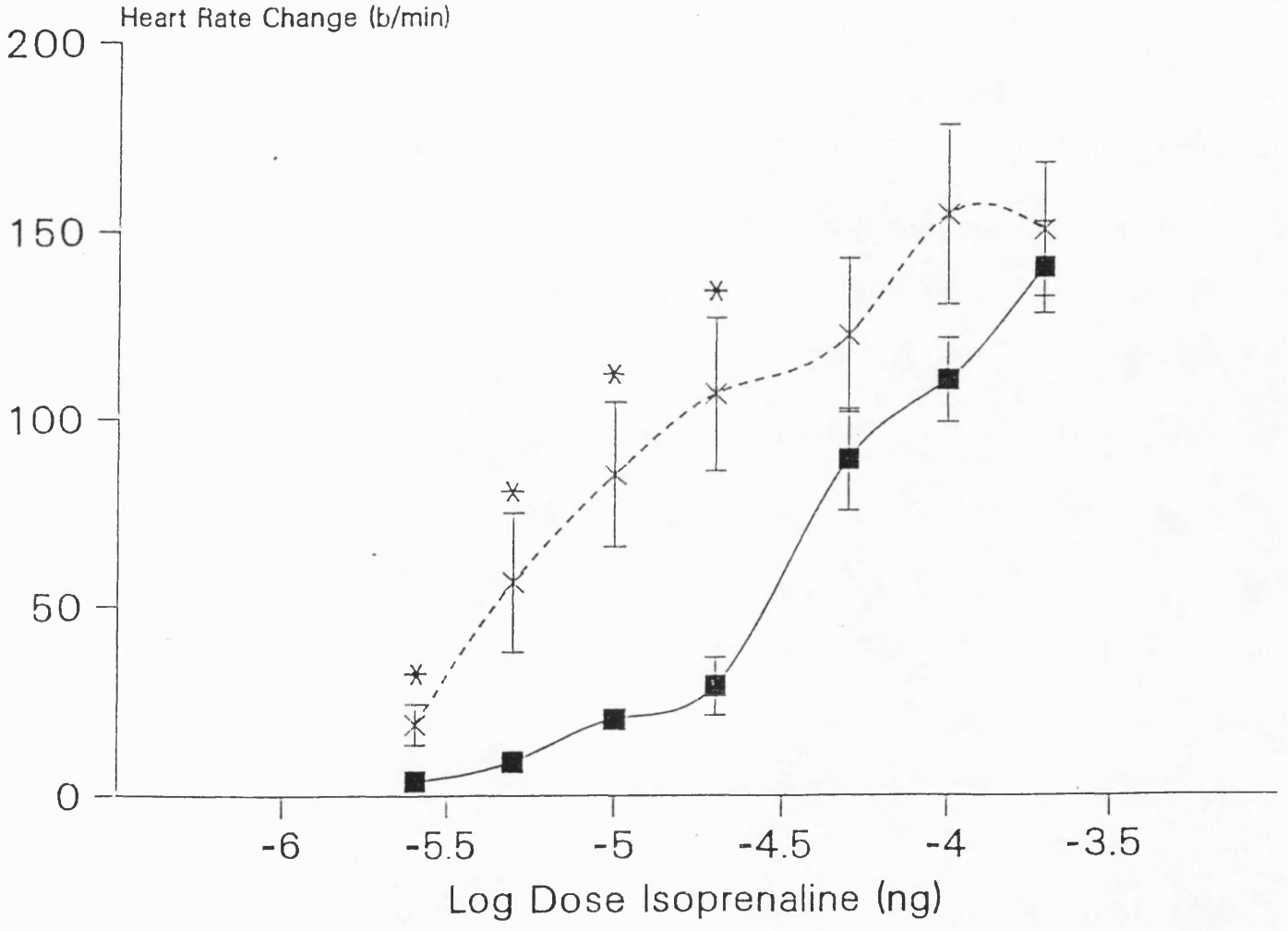


----- Nitrendipine (3mg/kg in 5% PEG 1ml/100g) p.o.; mean ±SE, n=4.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=4.

FIGURE 34

Graph showing the effect of 7 days pretreatment with nitrendipine (3mg/kg) p.o. on the heart rate response to exogenous isoprenaline in the anaesthetised Wistar rat.



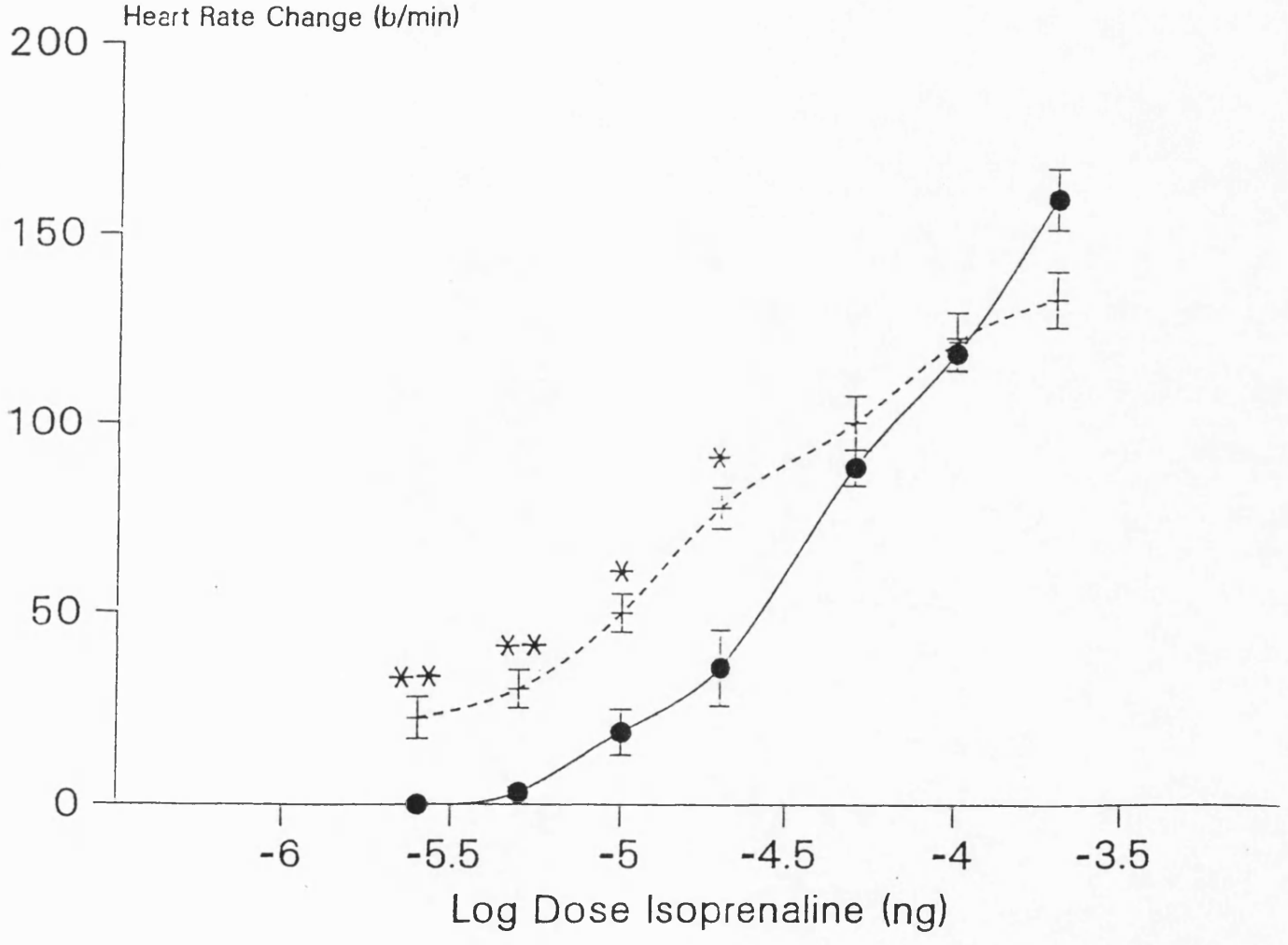
X----X Nitrendipine (3mg/kg) p.o. (n=4) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=8) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 35

Graph showing the effect of 21 days pretreatment with nitrendipine (3mg/kg) p.o. on the heart rate response to exogenous isoprenaline in the anaesthetised Wistar rat.



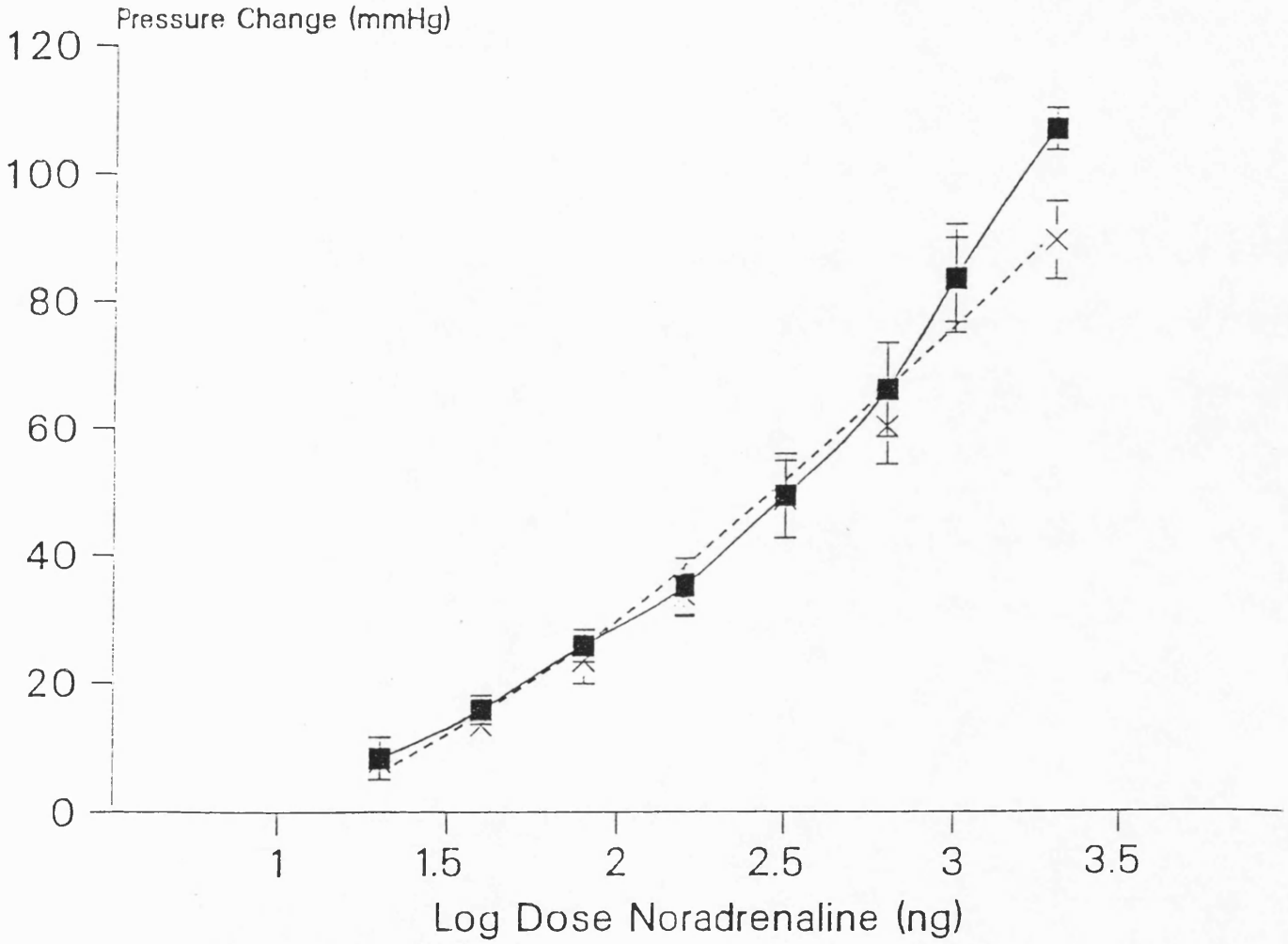
+----+ Nitrendipine (3mg/kg) p.o. (n=4) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=8) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 36

Graph showing the effect of 7 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Wistar rat.

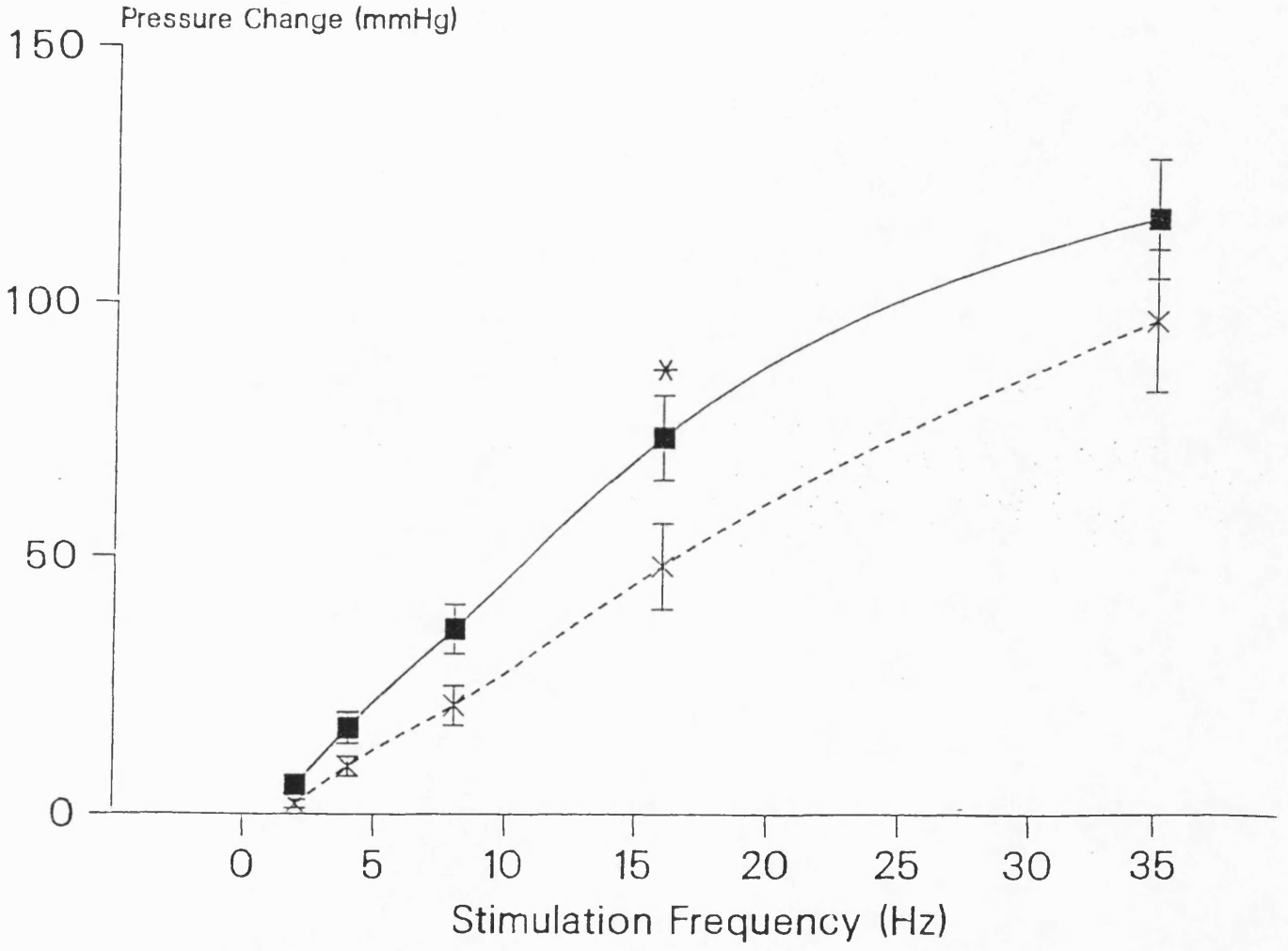


X----X Nitrendipine (3mg/kg) p.o. (n=7) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 37

Graph showing the effect of 7 days pretreatment with nitrendipine (3mg/kg) p.o.. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Wistar rat.

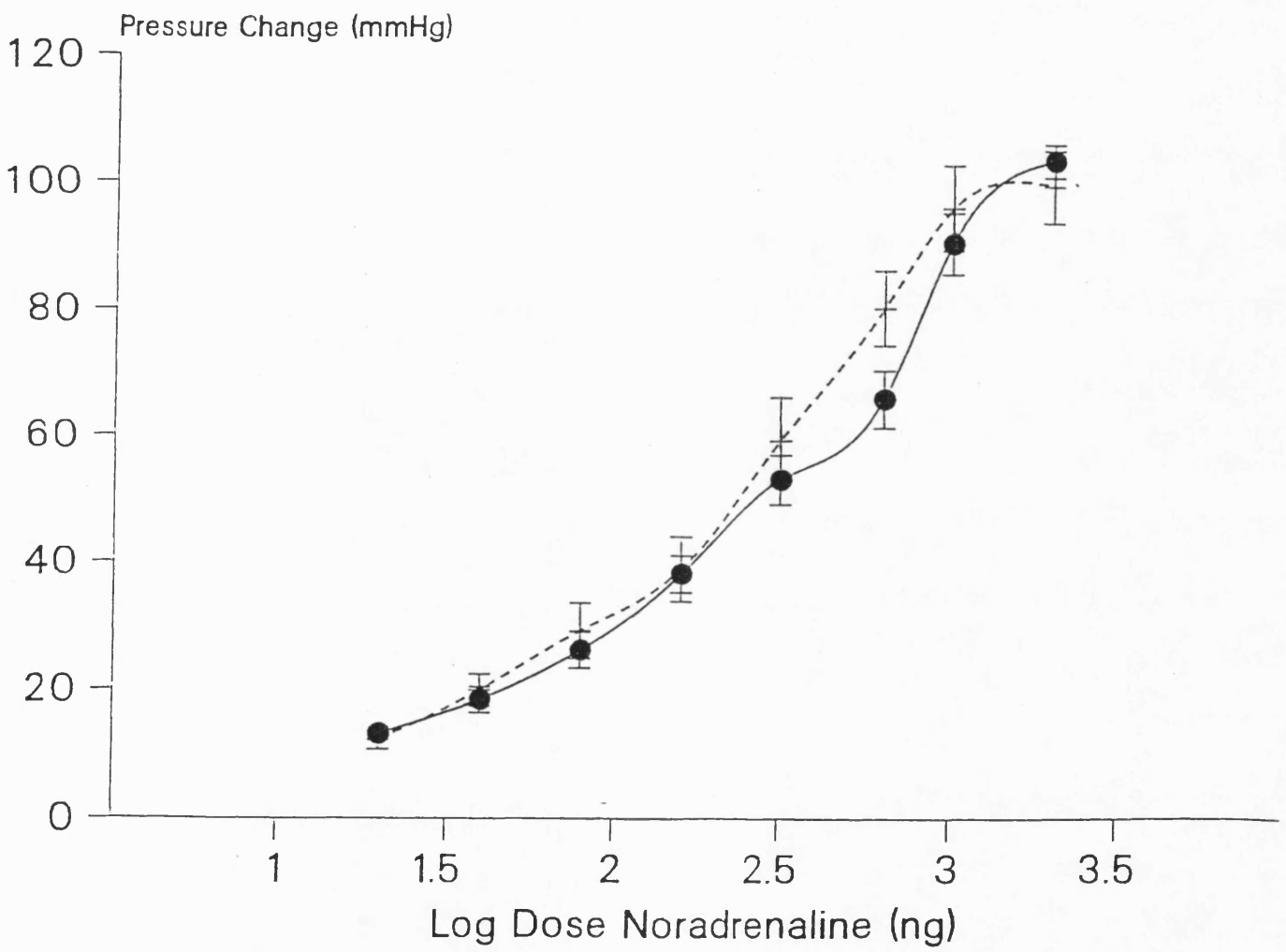


X----X Nitrendipine (3mg/kg) p.o. (n=7) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 38

Graph showing the effect of 21 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Wistar rat.

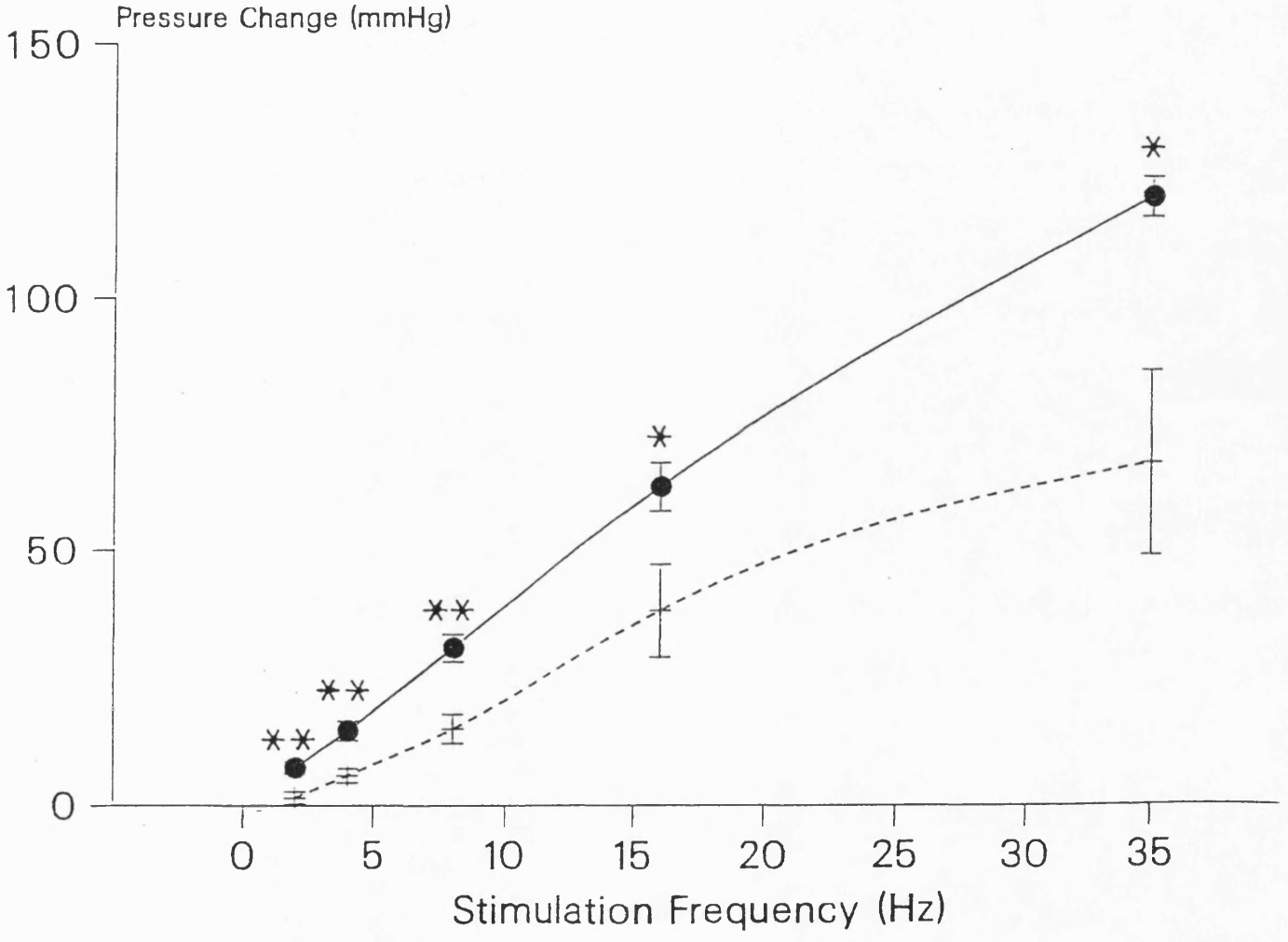


+----+ Nitrendipine (3mg/kg) p.o. (n=8) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=7) mean ±SE.

FIGURE 39

Graph showing the effect of 21 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Wistar rat.



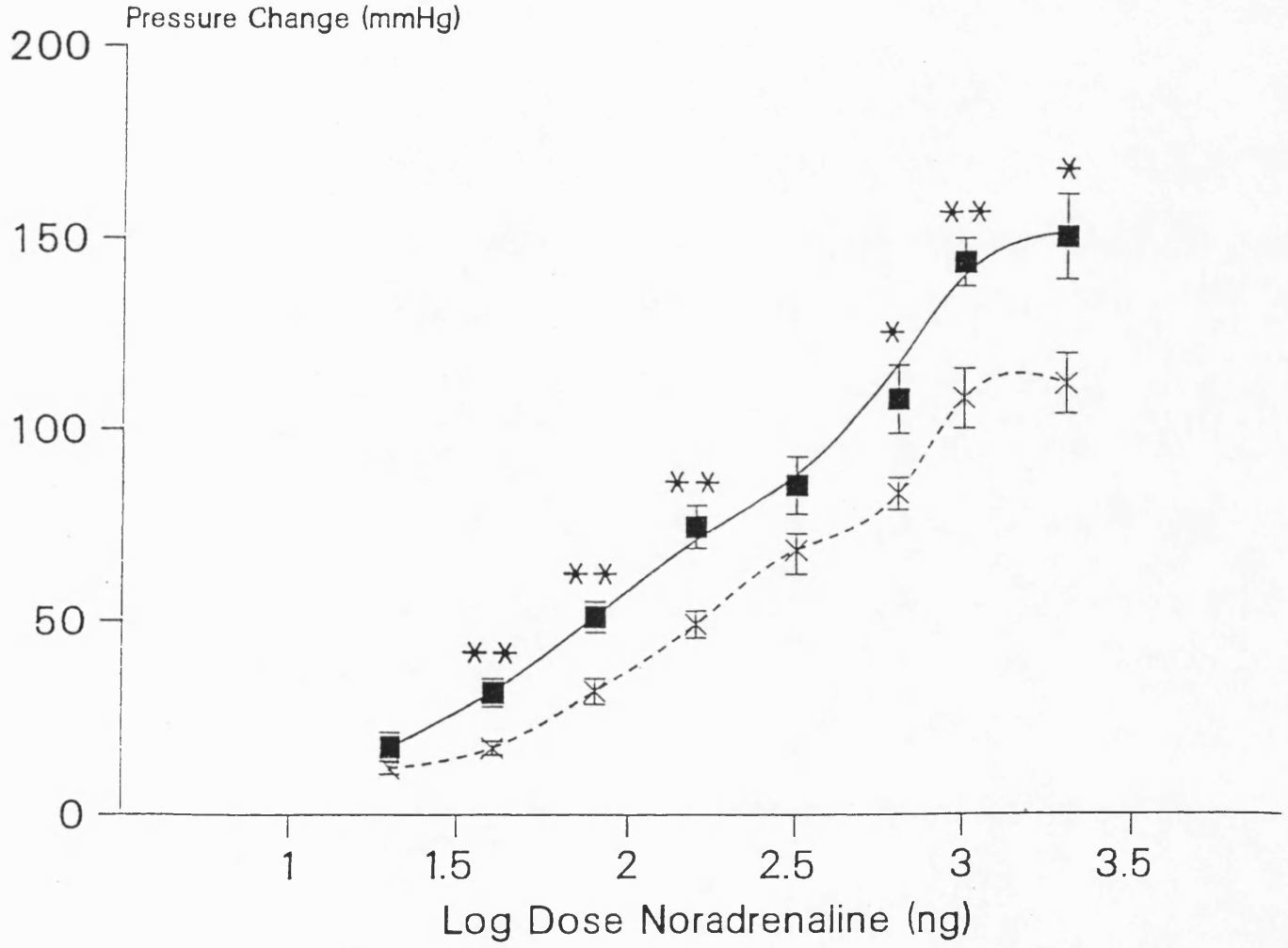
+----+ Nitrendipine (3mg/kg) p.o. (n=8) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=7) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 40

Graph showing the effect of 7 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Japanese Okamoto SHR.



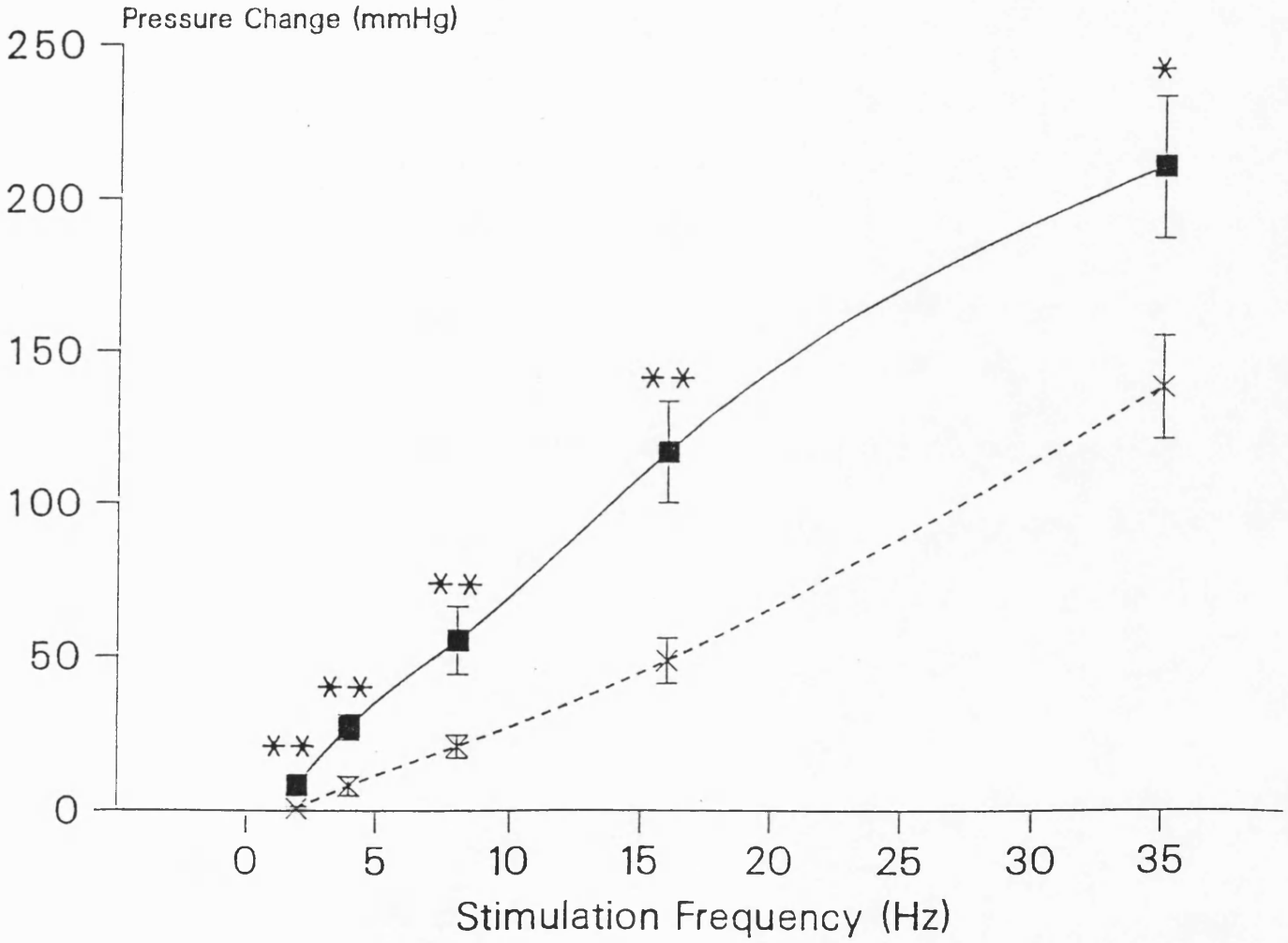
X----X Nitrendipine (3mg/kg) p.o. (n=8) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 41

Graph showing the effect of 7 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Japanese Okamoto SHR.



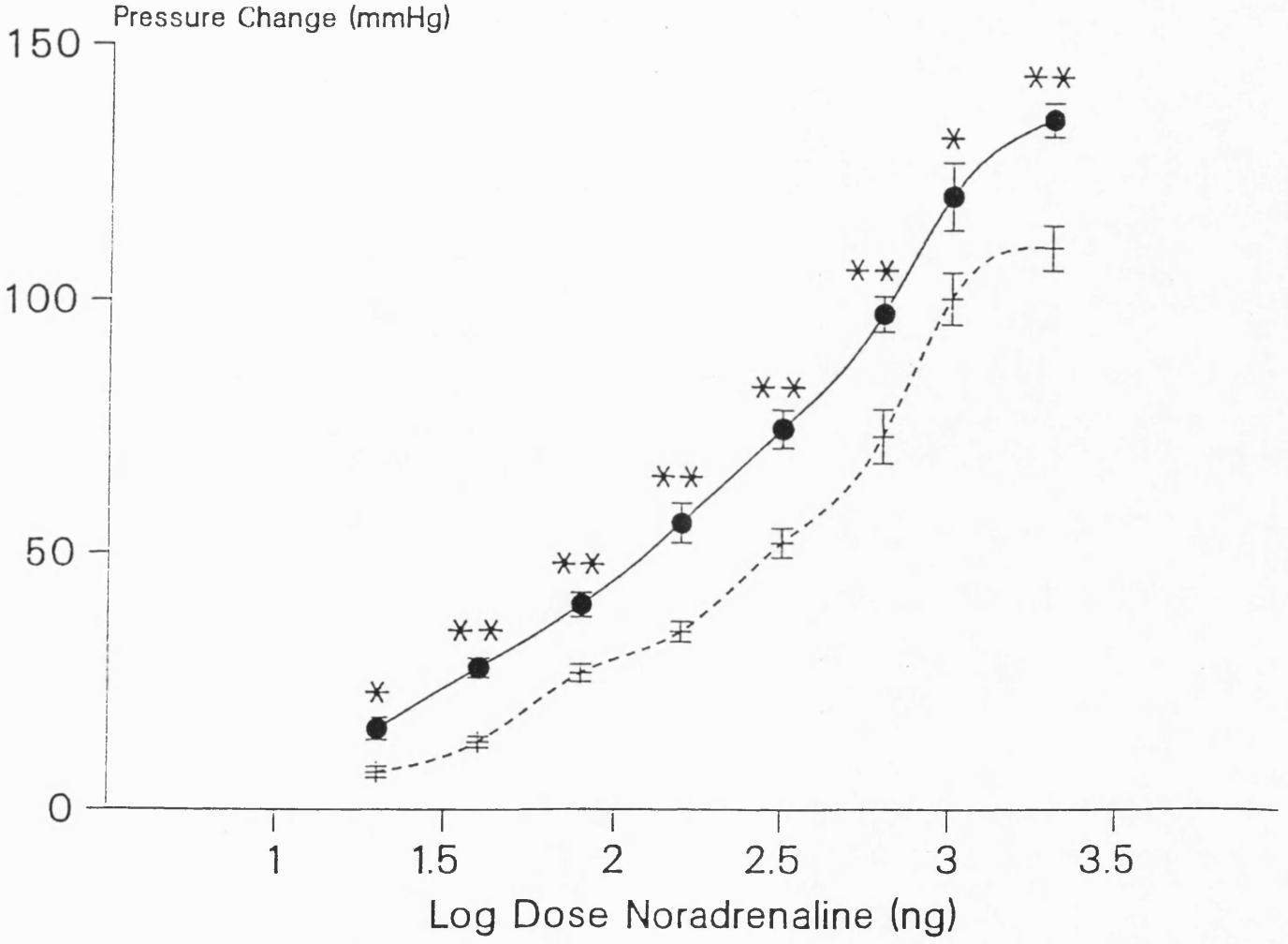
X----X Nitrendipine (3mg/kg) p.o. (n=7) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 42

Graph showing the effect of 21 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Japanese Okamoto SHR.



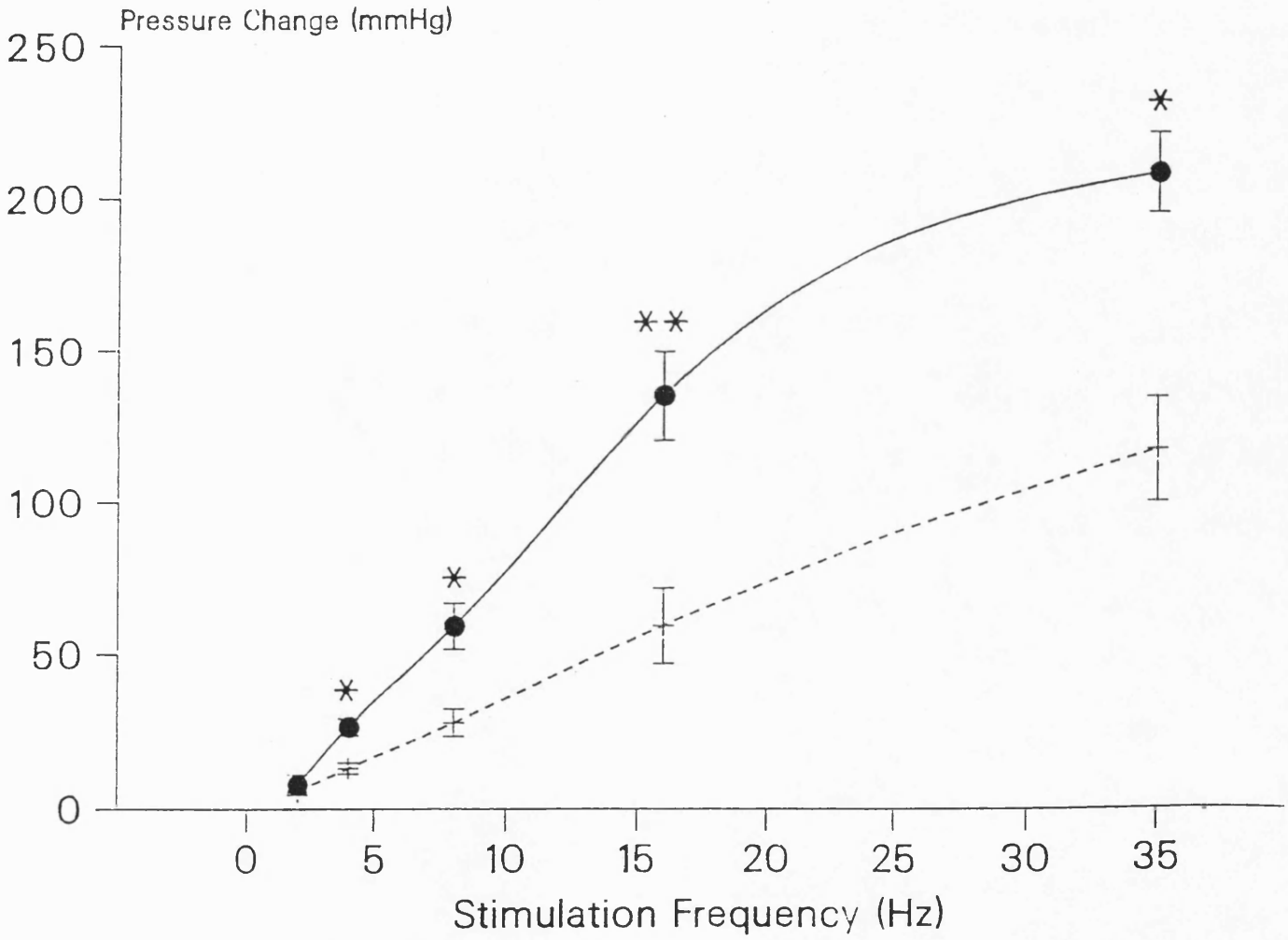
+----+ Nitrendipine (3mg/kg) p.o. (n=6) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 43

Graph showing the effect of 21 days pretreatment with nitrendipine (3mg/kg) p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Japanese Okamoto SHR.



+----+ Nitrendipine (3mg/kg) p.o. (n=6) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

3.4 CONCLUSIONS.

3.4.1 Conclusions from the conscious rat studies.

Treatment with nitrendipine (3 mg/kg) reduced the blood pressure in both normotensive and hypertensive rats. This hypotensive effect was accompanied by a reflex tachycardia in normotensive but not hypertensive animals. These results are shown in the graphs (figures 30-33) on pages 131-134.

The reduction of blood pressure followed a similar pattern in both normotensive and hypertensive animals, that is, pressure was reduced over the first 2-3 days of dosing. The blood pressure remained at this reduced level throughout the whole of the rest of the dosing period. There was no tendency for pressure to plateau at a higher level, as it did during atenolol treatment (section 2.4.1). There was, then, no evidence of any change in the antihypertensive effect of nitrendipine over the experimental period. The variation in blood pressure measured 2 and 24 hours after doses of nitrendipine decreased with time. The values were lowest 2 hours after dosing, a time that corresponds well to the expected peak plasma concentration of the drug (see section 3.1.5). The fact that this daily variation decreased with time suggests that nitrendipine reached more stable plasma concentrations with chronic dosing. It was interesting that nitrendipine treatment caused a reduction in blood pressure in normotensive animals. Previous work (Pegram *et al* 1984, Dunn *et al* 1984) has suggested that chronic nitrendipine administration does not produce a hypotensive effect in normotensive animals. The hypotensive effect of nitrendipine observed in the normotensive rats was only 30% of that obtained in

hypertensives, and significantly smaller than that observed in normotensives treated with atenolol (section 2.3.1(i)). This suggests that, as previously reported, the hypotensive effect of nitrendipine is much greater when the peripheral resistance is initially raised. The fact that a small hypotensive effect was observed in the normotensive animals may have been partly due to the fact that the physiological stress reaction to the measuring procedure may have raised the blood pressure. This "raised" blood pressure may have been reduced by nitrendipine.

The reflex tachycardia present on treating the normotensive rats was not apparent in the hypertensive animals; this has been described by other workers (Pegram *et al* 1984, Dunn *et al* 1984). This effect may be due to an abnormality in the baroreceptor activity in spontaneously hypertensive animals (Sapru & Wang 1976, Pfeffer & Frohlich 1973) preventing the reflex increase in heart rate. Alternatively, the physiological adaptation to both the stress of blood pressure measurement and to the warming involved may "swamp" any drug-induced tachycardia. Physiological responses to warming involve peripheral vasodilation and an associated tachycardia, while that to stress involves tachycardia as a direct sympathetic response. As animals become accustomed to the procedures, these effects would be expected to be reduced resulting in a reduction in tachycardia due directly to the measuring procedure. This would explain the reduction in heart rate over time observed in both control and treated SHRs (figure 33). The fact that this effect is not present in normotensive rats may be explained by the relative importance of the stress-induced tachycardia; SHRs are known to be especially susceptible to the effects of stress.

3.4.2 Conclusions from the assessment of β-adrenoceptor blockade.

The dose-response curves obtained to isoprenaline after both 7 and 21 days pretreatment with nitrendipine show that there is no sign of any β-adrenoceptor blockade. (figures 34 & 35)

The response to isoprenaline in the anaesthetised rats seems to have been increased following nitrendipine pretreatment. Low doses of isoprenaline have produced significantly greater increases in heart rate following pretreatment. There is no evidence that nitrendipine has any effect on cardiac β-adrenoceptors (section 3.1.3 & 4) so this response is somewhat unexpected. The explanation of this increased response lies with the preparation used for the investigation. Isoprenaline is a β-adrenoceptor agonist which acts at β_1-adrenoceptors in the heart to produce an increase in rate and at peripheral β_2-adrenoceptors producing vasodilation. In the whole animal preparation used to investigate β-adrenoceptor blockade both these systems were intact. The vasodilation produced by isoprenaline's β_2-adrenoceptor effect may be expected to contribute to the increase in heart rate via baroreceptor reflex activity. It would seem possible that in the presence of nitrendipine-induced vasodilation the vasodilatory effects of isoprenaline would be of a sufficient magnitude and duration to initiate the reflex tachycardia at lower doses. Higher doses of the β-adrenoceptor agonist would "swamp" this "additive" effect. This is a potential explanation for the increased response to isoprenaline at the lower end of the dose response curve. If this explanation is correct, an investigation of β-blockade using an Langendorff preparation would not show an increase in isoprenaline response.

3.4.3 Conclusions from the *in situ* blood perfused mesentery model.

The results from the investigation of adrenergic neurotransmission using the *in situ* blood perfused mesentery are shown in figures 36-43. There was no change in the responses of normotensive rats after 7 days nitrendipine pretreatment and only small changes after 21 days. After the longer nitrendipine treatment there was some evidence of a slightly reduced response to periarterial electrical stimulation. In the hypertensive animals nitrendipine pretreatment reduced responses to both noradrenaline and electrical stimulation after both 7 and 21 days.

The absence of any large change in mesenteric response in normotensive animals was consistent with the relatively small hypotensive effect obtained in the conscious animal. This supports the theory that nitrendipine only has an antihypertensive effect when the peripheral resistance is initially raised. In the hypertensive animals nitrendipine pretreatment reduced the response to both noradrenaline and electrical stimulation suggesting that its effect was postjunctional. This attenuation in response was large enough to account for the antihypertensive effect observed in the conscious animal. This is consistent with the work of Pedrinelli and Tarazi (1984) who showed that nitrendipine blocked the effects of both endogenous and exogenous noradrenaline. It also supports the idea that at concentrations achieved during clinical usage there is no indication of any presynaptic effect (Eikenburg & Lochandwala 1986). The results suggest that nitrendipine exerts its antihypertensive effect by blocking Ca^{++} flux associated with noradrenaline's interaction with postjunctional α-adrenoceptors. Eikenburg (1984) has demonstrated that both endogenous and exogenous noradrenaline cause vasoconstriction via postsynaptic interactions with predominantly α_1-

adrenoceptors in the *in situ* blood perfused mesentery. As nitrendipine pretreatment reduced these responses and had no apparent presynaptic effects it would seem that nitrendipine is able to inhibit the Ca^{++} flux associated with the α_1-adrenoceptor. This is consistent with the idea that nitrendipine can inhibit the Ca^{++} flux associated with both α_1- and α_2-adrenoceptors (Eikenburg & Lochandwala 1986, Pedrinelli and Tarazi 1985a, 1985b) and so inhibit the Ca^{++}-dependent contraction of vascular smooth muscle. By doing this it reduces vascular tone and thus reduces blood pressure by reducing peripheral resistance. The reduced noradrenaline response observed in the mesentery suggests that nitrendipine reduces mesenteric vascular tone and thus resistance. This supports the findings of Pegram and co workers (1984); and, as the mesentery is a large and important vascular bed, suggests that it is an important site for the reduction in systemic blood pressure.

3.4.4 Overall conclusions.

Nitrendipine treatment has been shown to reduce systemic blood pressure at the dose used, in both normotensive and hypertensive rats. The reduction in normotensive animals was smaller and associated with a reflex tachycardia. No apparent β-adrenoceptor blockade was produced by nitrendipine; and its hypotensive action appeared to be adequately explained by its antagonism of Ca^{++} flux associated with postjunctional α-adrenoceptors.

These findings are consistent with the theory that nitrendipine's main antihypertensive action is a blockade of Ca^{++} induced contraction in vascular smooth muscle and thus a reduction of peripheral resistance. They also support the idea that this effect is greater when peripheral resistance is initially high, such as in the spontaneously hypertensive rat.

These conclusions are more fully discussed and compared with those from other treatment groups in chapter 5.

CHAPTER 4.

COMBINATION

4.1 INTRODUCTION.

4.1.1 Historical context of combination treatment of hypertension.

During the past two decades, encouraging strides have been made in the recognition and treatment of high blood pressure and in the development of antihypertensive therapy. Despite increasing education about the risk-factors involved in cardiovascular disease and changing attitudes to health, there are still concerns surrounding hypertension and its control. As many as two thirds of the hypertensive patients in the USA remain inadequately controlled (Tarazi & Fouad-Tarazi 1986). The β-adrenoceptor blockers were heralded as the antihypertensive drugs of the sixties and early seventies. These drugs, used alone or in combination with thiazide diuretics, have led the assault on hypertension. Recently, the use of thiazide diuretics and β-blockade has been questioned. There is evidence that thiazide-induced hypokalaemia may lead to ventricular extrasystoles and so may increase the risk of myocardial infarction (Moser 1986). With the reduction in the use of thiazide diuretics there is room for increasing the use of calcium antagonists, both alone and in combination. Calcium antagonists are now rapidly establishing themselves as the antihypertensive drugs of the eighties.

4.1.2 Theoretical considerations for the use of combination treatment.

Before the possible benefits of the combined use of β-adrenoceptor blockers and calcium antagonists can be evaluated, their modes of action should be considered. The pharmacology of these two entirely different groups of drugs has been discussed in some detail in the introductions to chapters 2 and 3. The pharmacological basis of the antihypertensive action of atenolol and nitrendipine are discussed in sections 2.1.7 and 3.1.7 respectively.

On referring to the previous sections concerning the modes of action of atenolol and nitrendipine it can be seen that, although the exact mechanism of atenolol's hypotensive action is unknown, there is no evidence of it having any effect on voltage operated Ca^{++}-channels. Similarly there is no evidence of nitrendipine possessing any ability to blockade β-adrenoceptors. It is theoretically possible, therefore, that, as both these drugs produce a reduction in blood pressure via different mechanisms of action, their use in combination may prove additive.

The pharmacological profiles of nitrendipine and atenolol make them appear particularly well suited for combination therapy. While some calcium antagonists, such as verapamil, reduce AV conduction producing a potentially dangerous situation in the presence of β-blockade; nitrendipine has no effects on cardiac nodal tissue or conduction in the heart (Van Zweiten & Timmermans 1983, Rutsch & Schmutzler 1984, Maltz *et al* 1984). Indeed the presence of β-adrenoceptor blockade would have the advantage of reducing the reflex tachycardia observed with nitrendipine treatment (Warltier *et al* 1984). Nitrendipine would also counteract the reduction in coronary blood flow associated with the "unmasking" of α-adrenoceptors

during β-adrenoceptor blockade (Nayler 1988).

Nitrendipine's reduction of free Ca^{++} has been shown to inhibit atherosclerotic plaque formation and reduce platelet aggregation (Erne *et al* 1984). This is particularly beneficial as β-blockade has been shown to increase the low/high-density lipoprotein cholesterol ratio and so create conditions which favour plaque formation (Weinstein & Heider 1987, Mac Carthy 1988).

As nitrendipine undergoes rapid metabolism in the liver (section 3.1.5), interactions with a lipid soluble β-blocker, such as propranolol, which is similarly handled are possible. However, they are unlikely with atenolol which is not lipid soluble and is excreted by the kidneys after only minimal metabolism (section 3.1.5).

In the treatment of hypertension, patients who do not respond to β-blockers alone are often treated with the addition of a diuretic. From a theoretical standpoint, however, treatment with a combination of a β-adrenoceptor blocker and a calcium antagonist offers a better therapeutic rationale. Atenolol and nitrendipine seem particularly suitable for such a regime as they lack deleterious pharmacological and metabolic interactions and have positive interactions resulting in a reduced incidence of side-effects. These theoretical considerations have led to this combination of drugs being widely clinically investigated.

4.1.3 Clinical basis for the use of the combination therapy.

The combination of atenolol and nitrendipine was studied in a large multicenter trial in patients with mild to moderate hypertension (Kirkendall *et al* 1987). After an initial placebo period patients were

treated with two of the following three drugs: atenolol (50mg), nitrendipine (20mg) and hydrochlorothiazide (50mg). While all three regimes were effective at reducing blood pressure, the mean fall in systolic blood pressure was significantly greater in patients receiving the combination of atenolol and nitrendipine. This regime also resulted in a greater number of patients achieving their target blood pressure and fewer failures to respond.

The study of De Divitiis *et al* (1985) also used the combination of atenolol and nitrendipine and permitted the assessment of the hypotensive contribution of the individual agents to the overall effect. Significant additional blood pressure reduction was observed when atenolol (100mg/day) was added to treatment with nitrendipine (20mg/day). Further decreases in blood pressure were also obtained when patients with moderate hypertension, which persisted despite atenolol treatment, received additional nitrendipine. Also, as blood pressure was measured between 21 and 24 hours after the daily administration of drugs in this study, it suggests that the combination is effective when administered only once a day.

A study by De Kock *et al* (1985) showed that the combination of nitrendipine and atenolol was effective at improving early diastolic dysfunction in patients with essential hypertension. In all of these three studies using atenolol and nitrendipine in combination the incidence and severity of side-effects was found to be no greater, or less, than those experienced using the drugs individually. The incidence of tachycardia reported early in nitrendipine treatment was reduced in combination treatment and patients complained less of cold extremities during treatment with the combination than they did during treatment with atenolol alone.

Nitrendipine has also been investigated in combination with other β-adrenoceptor blockers. Mc Mahon (1986) reported on a multicentre trial with nitrendipine and propranolol involving 329 patients for over a year.

He concluded that the combination was safe, well tolerated and produced an additive effect on the reduction of blood pressure without a development of tolerance over the year. Maltz *et al* (1986) found that nitrendipine used in combination with propranolol did not affect atrioventricular conductivity and was a safe and potentially useful combination. The combination of nitrendipine and the β-blocker metoprolol also produced an additive hypotensive effect and reduced incidence of side-effects (Orö & Ryman 1984).

Similarly atenolol has been studied in combination with other dihydropyridine calcium antagonists. The combination of atenolol and nifedipine has been found to augment symptom benefit compared to either drug alone when used in treating both hypertension and chronic stable angina pectoris (Rowland *et al* 1983, Leon *et al* 1985). In general, clinical investigations involving the combined use of β-blockade and calcium antagonism have led to the conclusion that the drugs provide a more effective treatment in combination than either drug given alone (Bühler *et al* 1983, Mc Innes *et al* 1985, Mac Carthy 1988, Nayler 1988).

The high degree of antihypertensive action of the combination of atenolol and nitrendipine combined with the relatively low incidence of side-effects make it particularly useful in combatting severe hypertension. The pharmacokinetics of the drugs are such that effective therapy is provided by a single daily dose, a factor which increases patient compliance. These considerations suggest that the combination would provide better blood pressure control and improved quality of life.

The effect of the combination of atenolol and nitrendipine on blood pressure, heart rate and sympathetic neurotransmission together with their possible interactions was investigated in this work.

4.2 METHODS.

4.2.1 Investigation of blood pressure in the conscious rat.

Normotensive male Wistar rats and spontaneously hypertensive male Japanese Okamoto rats (approx. 200g, University of Bath strain) were used in this study. Systolic blood pressure was measured using a non-invasive "tail-cuff" technique. Full details of the protocol used are described in section 2.2.1 on page 33.

Blood pressure was measured before and two hours after the daily administration of drug. The treated animals received a combination of atenolol (50mg/kg) and nitrendipine (3mg/kg) in a 5% polyethylene glycol (PEG) vehicle. Control animals were dosed with the 5% PEG vehicle alone. During the first week of dosing all animals were dosed with PEG alone to allow the animals to become accustomed to the procedures involved. In the subsequent three weeks the treated animals were dosed with the combination and the control animals with PEG. Drugs were administered orally (1ml/100g) every day; blood pressure was not, however, measured during the weekend periods.

The investigation of the effects of chronic treatment with the combination of atenolol and nitrendipine and with the two drugs in isolation was carried out in a concomitant study.

4.2.2 Investigation of heart rate in the conscious rat.

The pulse pressure waves recorded during the measurement of systolic blood pressure were used to calculate heart rate. This was carried out with the data from both the normotensive and hypertensive rats.

Full details of this calculation procedure is shown in section 2.2.2 on page 34. As with the investigation of blood pressure this study was carried out in conjunction with the investigation of the two drugs in isolation.

4.2.3 Assessment of β-adrenoceptor blockade.

An anaesthetised rat preparation was used to assess the degree of β-adrenoceptor blockade. This was achieved by examining the effects of pretreatment on a dose-response curve obtained to bolus i.v. doses of isoprenaline.

The precise method used is explained in section 2.2.3 on page 35. This was carried out following 7 and 21 days pretreatment with either the combination of atenolol (50mg/kg) and nitrendipine (3mg/kg) or PEG control; in both cases drugs were administered orally (1ml/100g). The procedure was undertaken 24 hours after the final dose of either the combination or PEG.

4.2.4 The *in-situ* blood perfused mesentery method.

The effects of the combination of chronic β-adrenoceptor blockade and chronic calcium channel antagonism on adrenergic neurotransmission was investigated using the *in-situ* blood perfused mesentery method described by Jackson and Campbell (1980a). Details of the methodology is described in section 2.2.4 and shown as a diagram on page 39 (figure 3).

The protocol shown in section 2.2.4 was used to investigate mesenteric responses to both exogenous noradrenaline (20-2000ng in 0·9% w/v NaCl) and to periarterial electrical stimulation (15v rectangular pulses of 1ms duration for 20s, 2-35Hz). This was undertaken 24 hours after the last dose of either 5% PEG (1ml/100g) or the combination of atenolol (50mg/kg) and nitrendipine (3mg/kg) after 7 and 21 days pretreatment. This work was carried out using both normotensive male Wistar and spontaneously hypertensive male Japanese Okamoto rats. (University of Bath strain, approx 300-330g).

4.2.5 General considerations.

4.2.5(i) Animal husbandry.

The rats used in this study were housed at the University of Bath with a 12 hour light/dark cycle at an ambient temperature of 20-22°C and a relative humidity of 40-50%. Normotensive Wistar rats and spontaneously hypertensive Japanese Okamoto rats were used and were provided with

'labsure's' CRM diet and tap water *ad libitum* and were housed in groups so as to avoid both overcrowding and solitude.

Details of animal husbandry are described in more detail in section 2.2.7(i) on page 59.

4.2.5(ii) Statistical analysis.

As previously described blood pressure and heart rate data was analysed using the measurements recorded before the daily dose of either the combination or PEG. The distribution of the data was compared with the normal distribution using the Kolmogorov-Smirnov procedure and was checked by overlaying a normal distribution curve over a frequency histogram plotted from the data. An ANOVA with LSD, HSD and Scheffe's test follow-ups were carried out on the data collected during week I when all animals were treated with PEG alone. This was used to compare groups before any administration of "active" drug. The ANOVA and follow-up procedures were repeated on the data collected during week IV after three weeks treatment with the combinations. The ANOVA was used to examine the differences between all the groups, control, and atenolol and nitrendipine both alone and in combination. Unpaired Student's t-tests or Mann-Whitney U tests were used to compare two samples means depending on whether the data was non-parametric or normal.

In all experiments animals were assigned to groups in a random manner and treatment was randomly allotted to these groups. The statistical analysis was undertaken on a main-frame computer using the SPSS-X package.

Full details of the statistical analysis are described in section 2.2.7(iii) on page 62.

4.2.5(iii) Drugs and general chemicals.

The drugs and chemicals used are given in sections 2.2.7(iv) and 3.2.5(iii).

Atenolol was made up in a 5% PEG solution at a concentration of 10mg/ml and nitrendipine in PEG at 60°C and diluted to a 5% solution with the nitrendipine at a concentration of 0·6mg/ml. These two solutions were then mixed to give a solution of atenolol and nitrendipine at concentrations equal to those used during their individual dosing regimes. Animals all received the same relative volume of drug (1ml/100g) irrespective of whether they received atenolol, nitrendipine or the combination.

4.3 RESULTS.

4.3.1 Results of the investigation of blood pressure.

4.3.1(i) Results from conscious normotensive Wistar rats.

Throughout week I of the study when all the animals were dosed with 5% polyethylene glycol (PEG) alone, there was no significant difference in blood pressure between the two groups of animals. The mean systolic blood pressure of both groups of animals was elevated during the first day of the procedure; this then stabilised at a lower level (around 152mmHg) by the middle of the week.

Both groups of animals had a mean systolic blood pressure of 155mmHg (±1·7mmHg control, ±2·1mmHg combination) immediately prior to dosing with the "active" treatment in week II. During the first week of treatment with atenolol and nitrendipine in combination the mean systolic blood pressure of the treated group fell to a minimum of 122mmHg (± 5·1) while that of the PEG treated control group remained around 153mmHg (1·5). This large reduction in blood pressure observed in animals treated with the combination was of a similar magnitude and pattern as that described for atenolol-treated animals. A large and significant reduction in blood pressure became established during the first three days of treatment with the combination, falling to a minimum at the end of the week. The blood pressure of the group treated with the combination remained reduced compared with the control group but stabilised at the slightly higher level of around 143mmHg over the subsequent two weeks of treatment. The blood pressure of the control group remained fairly constant over the whole

experimental period; during week IV this was 153 (±1·6)mmHg. The mean systolic blood pressure of the group treated with the combination was 142 (±2·0)mmHg over the same period. Statistical analysis of the results obtained during the final week of dosing (week IV) showed a very highly significant difference between treatment groups ($p<0·0001$). Further analysis showed there was no difference between groups treated with atenolol and combination, while there was some evidence to suggest (Fisher's LSD, $p<0·05$) that these two groups were different from the group treated with nitrendipine alone. There was a very clear and large difference between all the treated groups and the control group.

The variation in blood pressure measured before and after dosing with the combination followed a similar pattern to that observed with atenolol treatment alone. The value measured two hours post-dosing was reduced; this reduction became less pronounced over time.

The graph on page 171 shows the effect of treatment with the combination on the systolic blood pressure of the normotensive male Wistar rat (Figure 44).

4.3.1(ii) Results from conscious spontaneously hypertensive rats.

During week I (all animals treated with PEG control) of the investigation there was no significant difference between the blood pressure of the two groups. The groups had mean systolic blood pressures of 214 (±5·7)mmHg and 211 (±3·0). As previously described (section 2.3.1(ii)) the systolic blood pressure of the hypertensive rats was found

to be higher than that of their normotensive counterparts during control PEG treatment.

Dosing with the combination of atenolol and nitrendipine began at the start of week II. The blood pressure of animals treated with the combination was greatly reduced compared with the control group over the first week of dosing. This reduction was large and maintained over the whole period of dosing. Blood pressure was not, however, reduced to normotensive levels. The blood pressure of the control group remained fairly constant over the whole period and was 227 (±1·3)mmHg over the final week of dosing. The blood pressure of the group treated with the combination was reduced to 199 (±2·1)mmHg over the same period. An ANOVA found this to be a highly significant reduction ($p<0·0001$) and follow-up procedures found no difference between groups treated with atenolol, nitrendipine or the combination. These groups were, however significantly different from the control group. As with the reduction in blood pressure observed with atenolol and nitrendipine alone, treatment with the combination produced a larger attenuation in the hypertensive rat than in its normotensive counterpart.

The variation in blood pressure measured pre- and post-dosing followed a similar pattern to that previously described. The variation was initially large but decreased over time; as before, this variation was larger in hypertensive animals.

Figure 45 on page 172 is a graph of the systolic blood pressure response of conscious hypertensive rats to the combined atenolol and nitrendipine treatment.

4.3.2 Results of the investigation of heart rate.

4.3.2(i) Results from conscious normotensive rats.

The graph on page 173 (Figure 46) shows the heart rate response of normotensive rats to treatment with the combination of atenolol and nitrendipine. There was found to be no significant difference between treatment groups during week I when all animals received PEG alone. The mean heart rate of the two groups over the first week was 446 (±12·0)b/min and 461 (±4·3)b/min respectively.

During the period of dosing with the combination (weeks II-IV) the heart rate of the control group slowly declined from 461 b/min to stabilise at around 400 b/min. The heart rate of the group treated with the combination decreased from 446 b/min to a minimum of 325 b/min after ten days dosing. This then increased slightly to around 367 (±2·2)b/min in the final week. This reduction was found to be statistically significant ($p<0·05$) using an ANOVA. Follow-up procedures show that there is no difference between the groups treated with atenolol alone or in combination, but that these groups are significantly different from both control and nitrendipine treated groups (which are themselves significantly different).

4.3.2(ii) Results from conscious hypertensive rats.

The results obtained from hypertensive rats treated with the combination of atenolol and nitrendipine are similar to those of rats treated with atenolol alone. Figure 47 on page 174 is a graphical representation of these results.

During week I (control) there was no significant difference between the heart rate of the two groups, both exhibited a gradual decline which continues throughout the whole treatment period. On commencing "active" treatment with the combination in week II the mean heart rate of the treated group was reduced compared to the control group. During the final week of dosing (week IV) the mean heart rate for the group treated with the combination was 331 (±5·9)b/min while that of the control group was 372 (±6·9)b/min, this difference was significant ($p<0·001$). There was found to be no statistical difference in heart rate between groups treated with atenolol alone or in combination.

4.3.3 Results of the assessment of β-adrenoceptor blockade.

Pretreatment with the combination of atenolol and nitrendipine for 7 days resulted in a displacement of the isoprenaline dose response curve to the right. This displacement is similar to that observed after pretreatment with atenolol alone. The lower portion of the curve is unchanged while there is a slight shift in the upper portion with a reduced

maximal response. Figure 48 on page 175 shows the two dose response curves.

A similar series of changes in isoprenaline dose-response curve was observed after 21 days pretreatment with the combination (Figure 49). The shift appeared to be more parallel after 21 days pretreatment with a significant ($p<0{\cdot}001$) attenuation of the upper half of the dose response curve. The β-adrenoceptor blockade observed after 7 days pretreatment with the combination appeared to be still present after 21 days.

4.3.4 Results from the *in-situ* blood perfused mesentery model.

4.3.4(i) Results from normotensive Wistar rats.

The dose-response curves obtained to exogenous noradrenaline following 7 days pretreatment with either the combination of atenolol and nitrendipine or 5% PEG are shown in figure 50 on page 177. There is clearly no difference in mesenteric response to exogenous noradrenaline following 7 days pretreatment with the combination.

Figure 51 (page 178) shows the frequency-response curve to periarterial electrical stimulation following 7 days pretreatment with the combination. There is evidence of a reduced response to low frequency stimulation (4 and 8Hz) following pretreatment with the combination.

However, there is no clear overall difference between the curve obtained from the animals treated with the combination and the control curve.

After 21 days pretreatment with the combination there is evidence of a reduced response to exogenous noradrenaline. The reduced response is only evident at either end of the curve, with an attenuated response to 20ng and 40ng noradrenaline and a reduced maximum response. These curves are shown in figure 52 on page 179.

Pretreatment with the combination for 21 days resulted in large and significant changes in the frequency response curve. There is no change in response to low frequency stimulation (2-8Hz). The response to stimulation at 16Hz was increased by 61%, and that to 35Hz by 55%. This increase in response was found to be highly statistically significant ($p<0.001$). This is shown in figure 53 on page 180.

The mean systemic blood pressure, mean mesenteric blood pressure and mesenteric resistance was measured in anaesthetised animals before the mesenteric responses to exogenous noradrenaline and periarterial electrical stimulation. These are shown in appendix 2. There appears to be a slight reduction in all of these parameters following 7 days pretreatment with the combination. This reduction is, however, no longer apparent after 21 days pretreatment.

4.3.4(ii) Results from spontaneously hypertensive rats.

The dose response curves to exogenous noradrenaline obtained after 7 days pretreatment with either the combination or PEG are shown in figure 54 on page 181. There is a large (40-50%) reduction in the responses to exogenous noradrenaline following pretreatment with the combination. This shift in the noradrenaline dose response curve was found to be highly statistically significant ($p<0.001$).

There was however, no significant change in the stimulation response curve obtained from animals pretreated with the combination for 7 days. This graph is shown in figure 55 on page 188 and there is clearly no difference in the curves obtained from "treated" and control animals.

The large and highly significant decrease in mesenteric response to exogenous noradrenaline observed after 7 days pretreatment with the combination is still present after 21 days pretreatment. This highly significant ($p<0.001$) shift in the noradrenaline dose response curve is shown in figure 56 on page 183.

There is no apparent change in response to periarterial electrical stimulation following 21 days pretreatment with the combination. This is similar to the situation after 7 days pretreatment, described above. The stimulation response curves are shown in figure 57 on page 184.

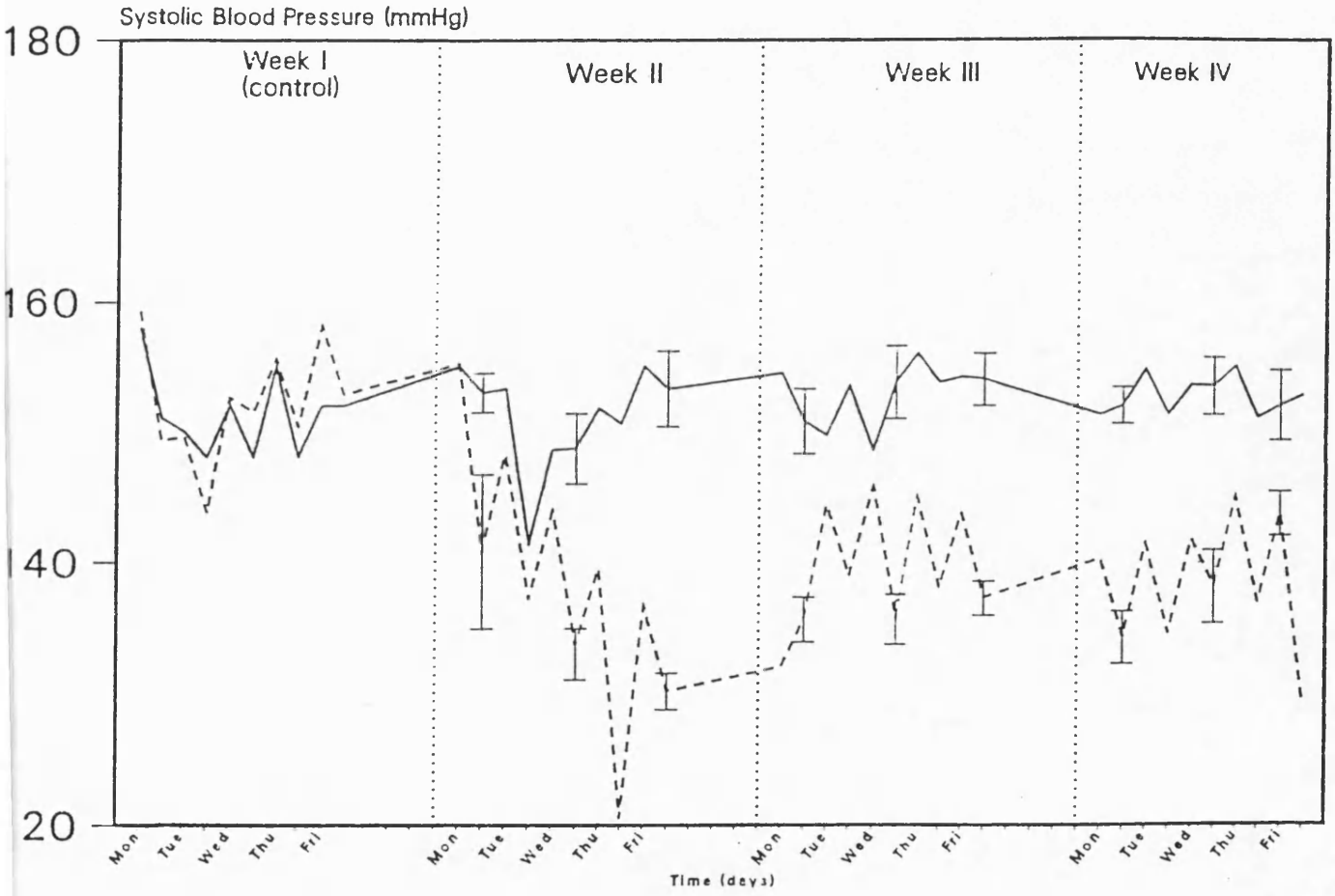
The mean systemic and mesenteric blood pressure and mesenteric resistance measured in the anaesthetised animal (appendix 2) were slightly reduced following 21 days pretreatment.

In the following graphs (figures 44-57), statistical difference was calculated between the values from control animals (treated with PEG) and the corresponding values from nitrendipine treated animals. This difference is shown by the following notation :

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE 44

Graph showing the effect of the combination (atenolol 50mg/kg, nitrendipine 3mg/Kg) p.o. on systolic blood pressure in the conscious normotensive male Wistar rat.

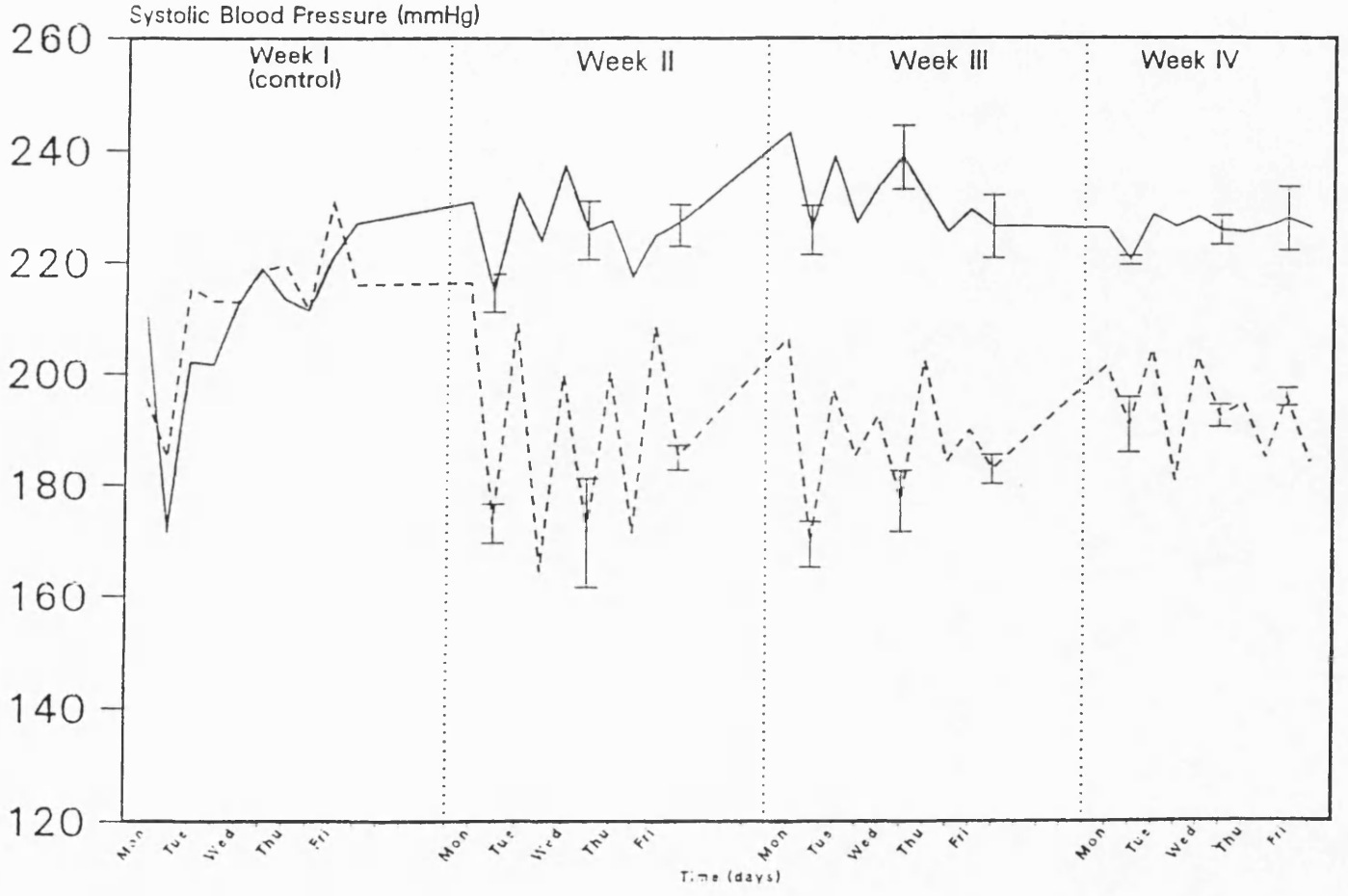


----- Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o.; mean ±SE, n=8.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=6.

FIGURE 45

Graph showing the effect of the combination (atenolol 50mg/kg, nitrendipine 3mg/Kg) p.o. on systolic blood pressure in the conscious spontaneously hypertensive male Japanese Okamoto rat.

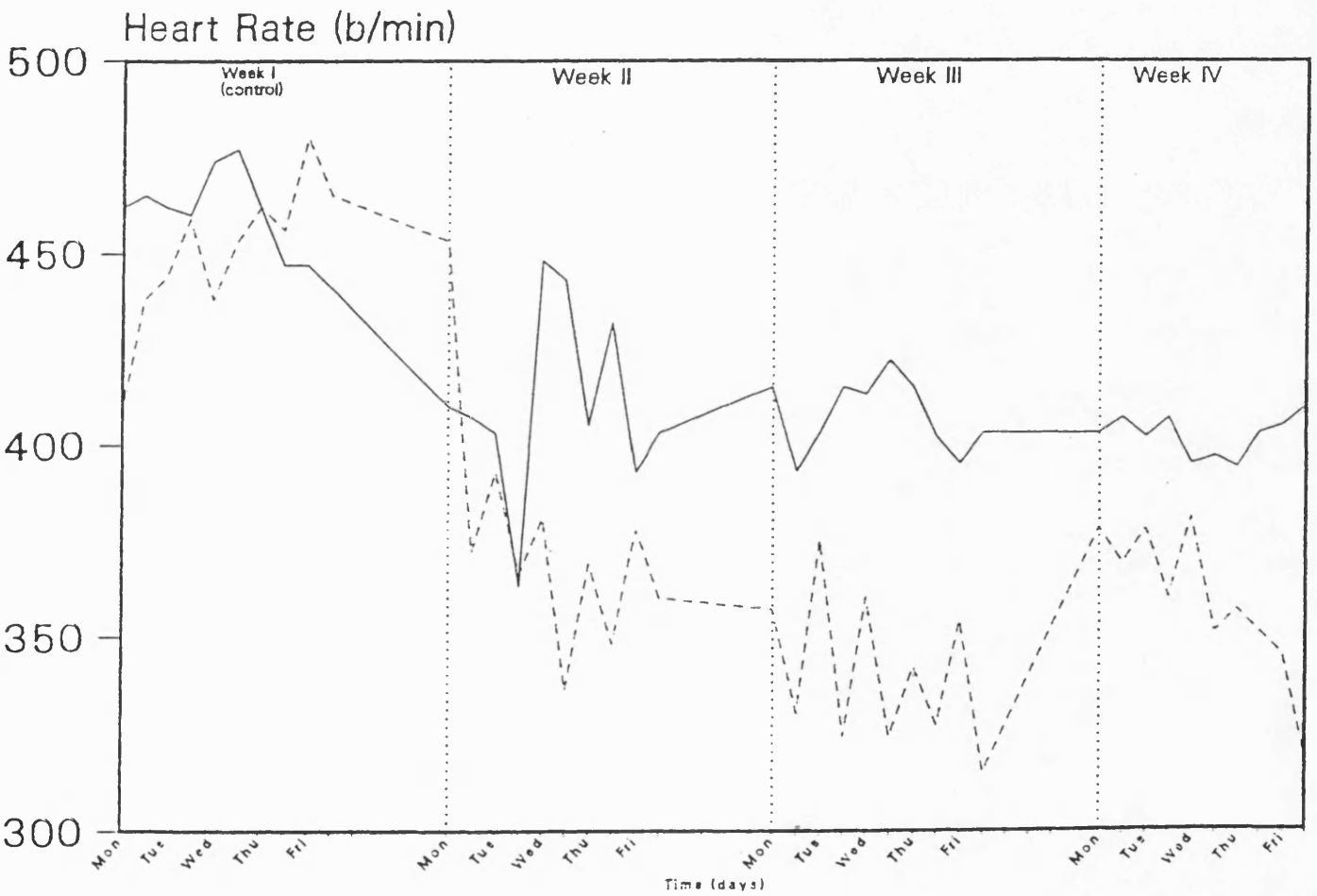


----- Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o.; mean ±SE, n=4.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=4.

FIGURE 46

Graph showing the effect of the combination (atenolol 50mg/kg, nitrendipine 3mg/Kg) p.o. on heart rate in the conscious normotensive male Wistar rat.

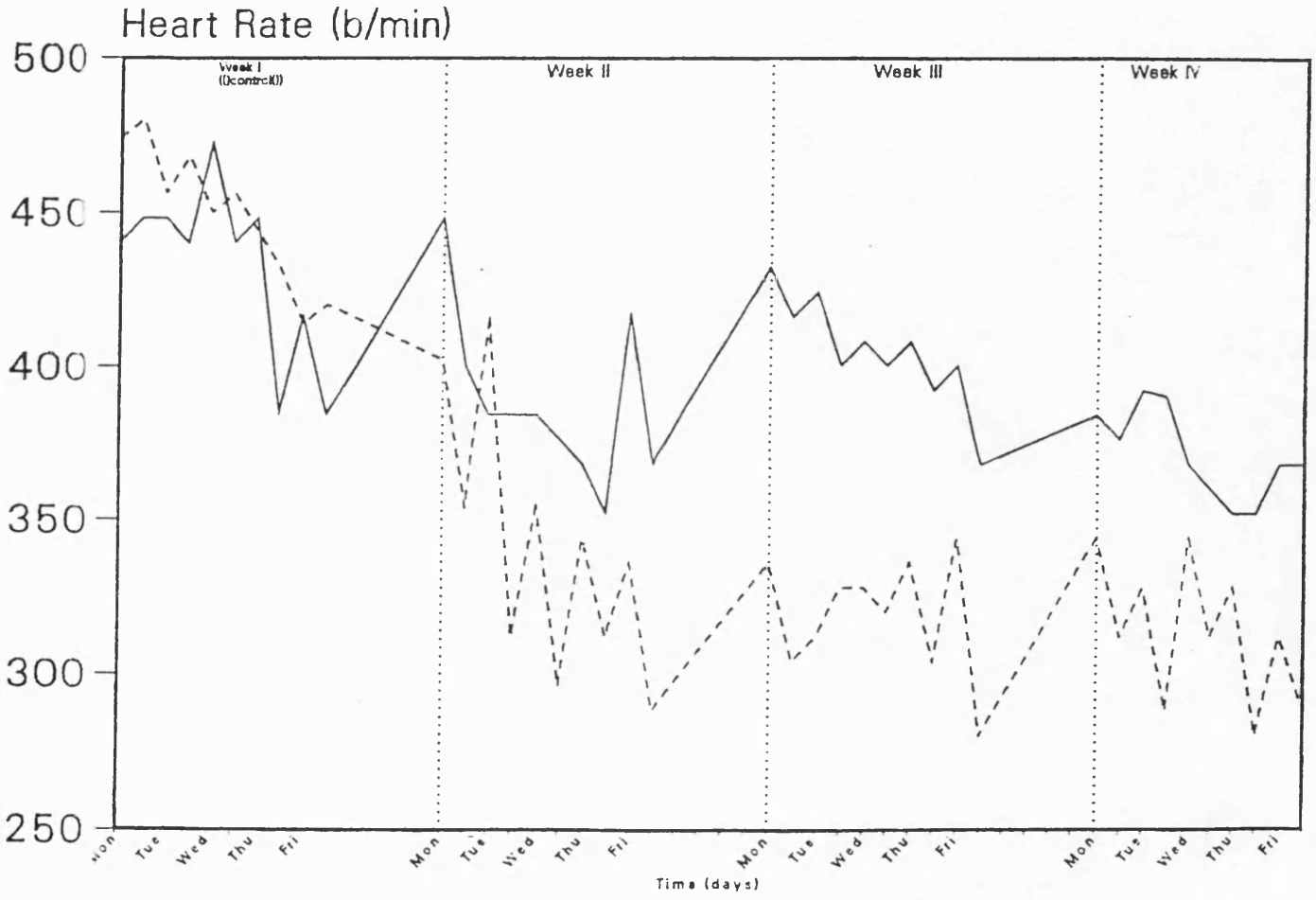


----- Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o.; mean ±SE, n=8.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=6.

FIGURE 47

Graph showing the effect of the combination (atenolol 50mg/kg, nitrendipine 3mg/Kg) p.o. on heart rate in the conscious spontaneously hypertensive male Japanese Okamoto rat.

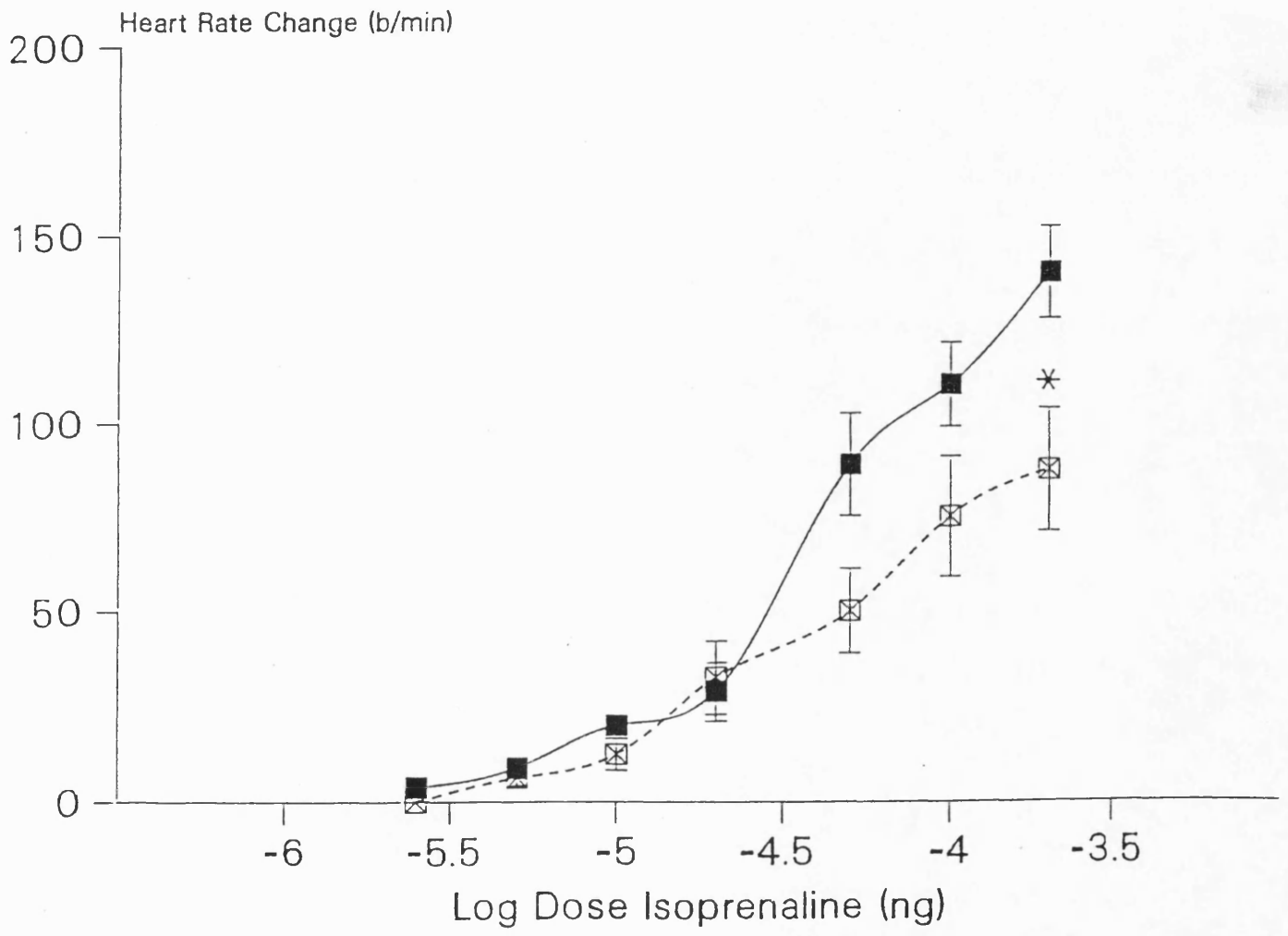


----- Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o.; mean ±SE, n=4.

——— Polyethylene glycol (5%, 1ml/100g) p.o.; mean ±SE, n=4.

FIGURE 48

Graph showing the effect of 7 days pretreatment with the combination p.o. on the heart rate response to exogenous isoprenaline in the anaesthetised Wistar rat.



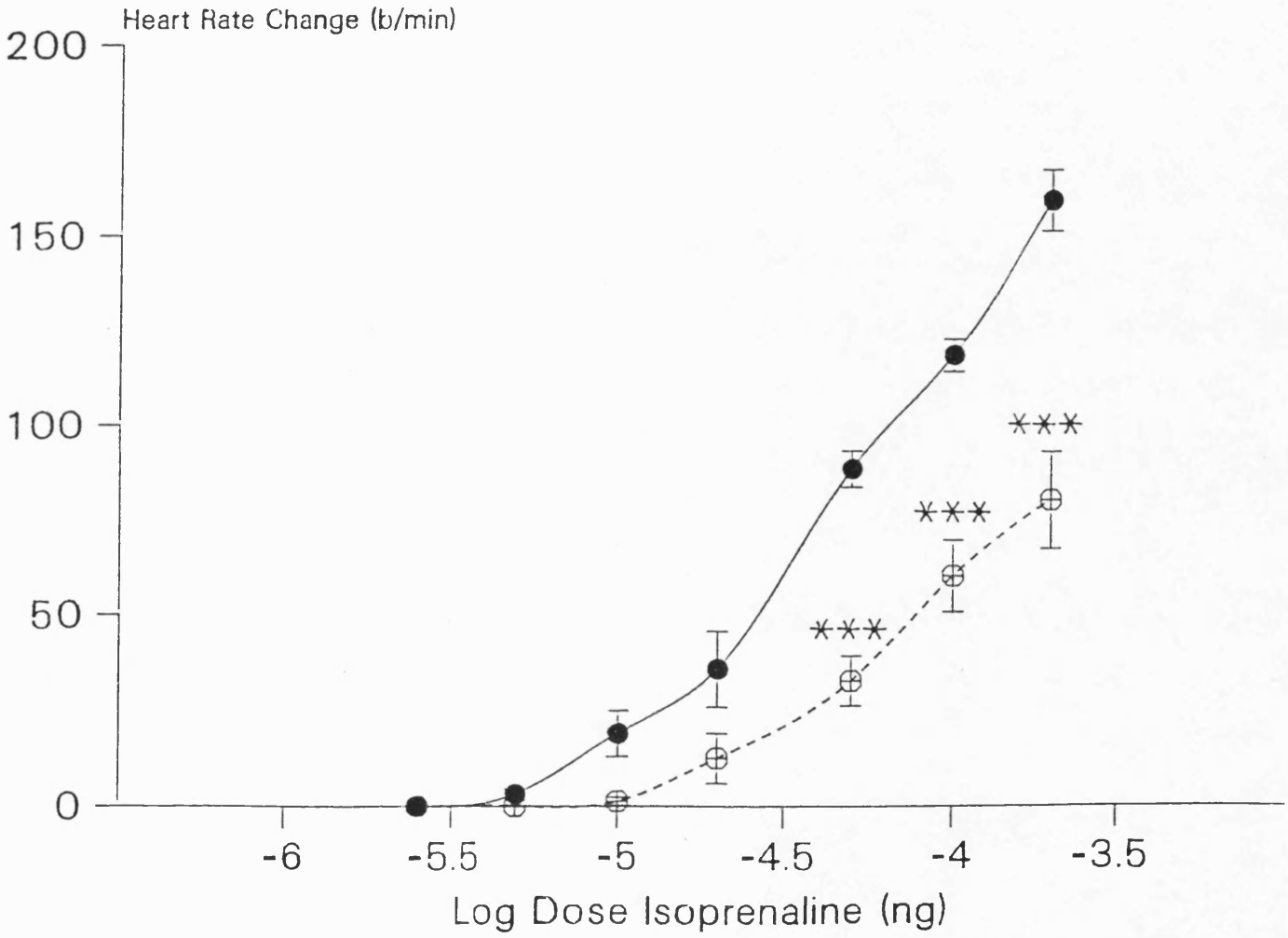
⊠----⊠ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=4) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=8) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 49

Graph showing the effect of 21 days pretreatment with the combination p.o. on the heart rate response to exogenous isoprenaline in the anaesthetised Wistar rat.



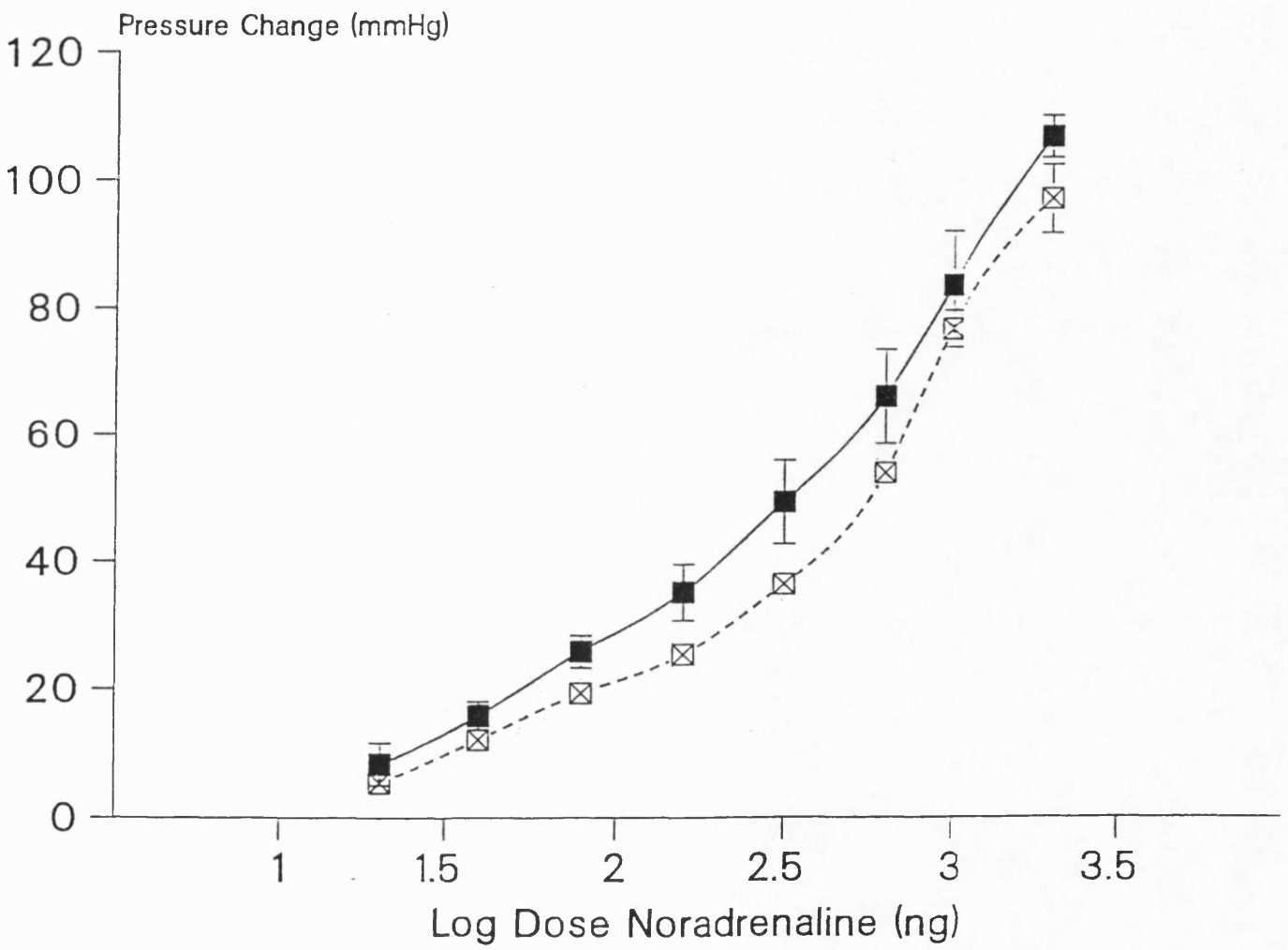
⊕----⊕ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=4) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=8) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 50

Graph showing the effect of 7 days pretreatment with the combination p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Wistar rat.

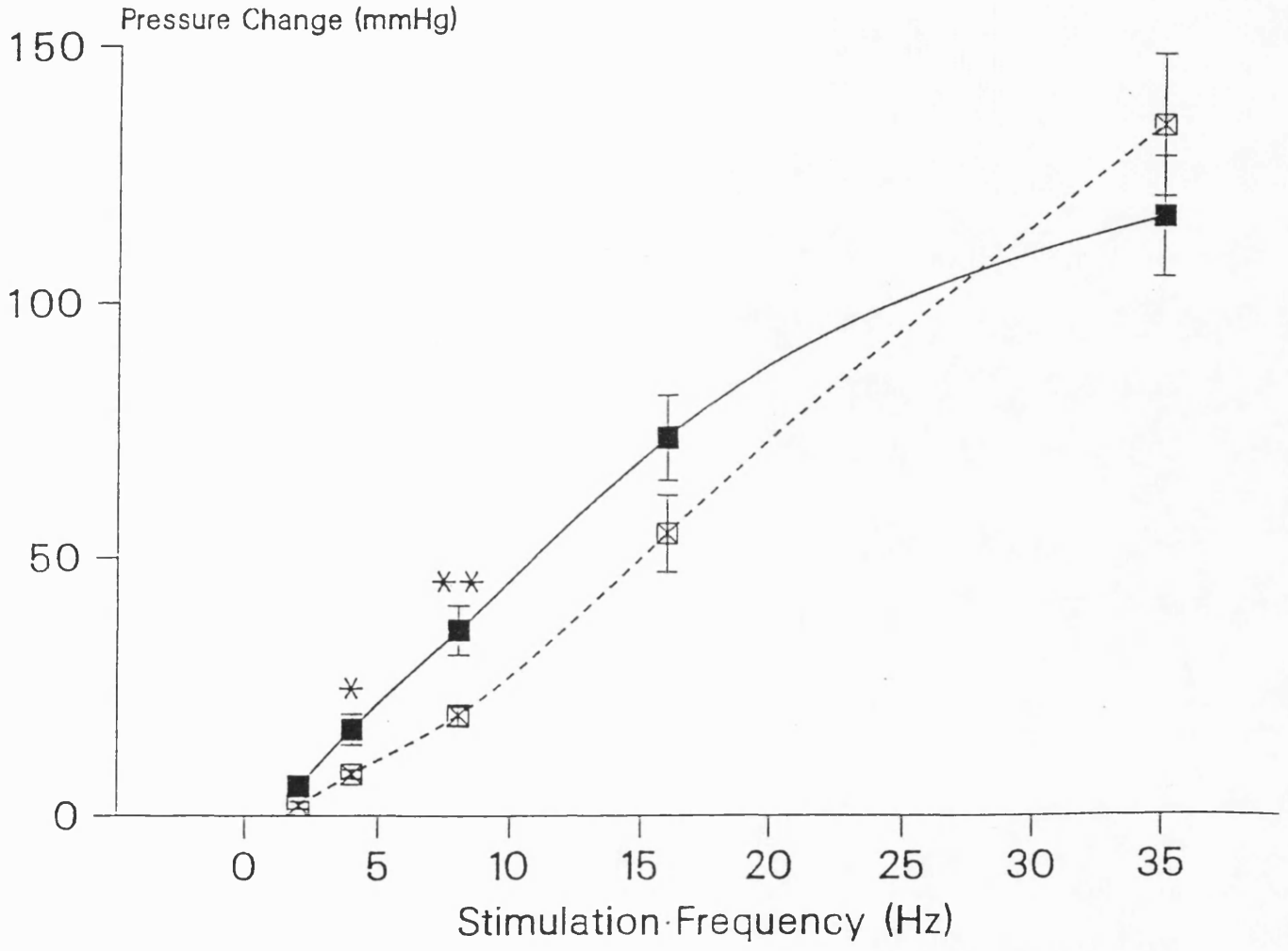


⊠----⊠ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=8) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 51

Graph showing the effect of 7 days pretreatment with the combination p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Wistar rat.

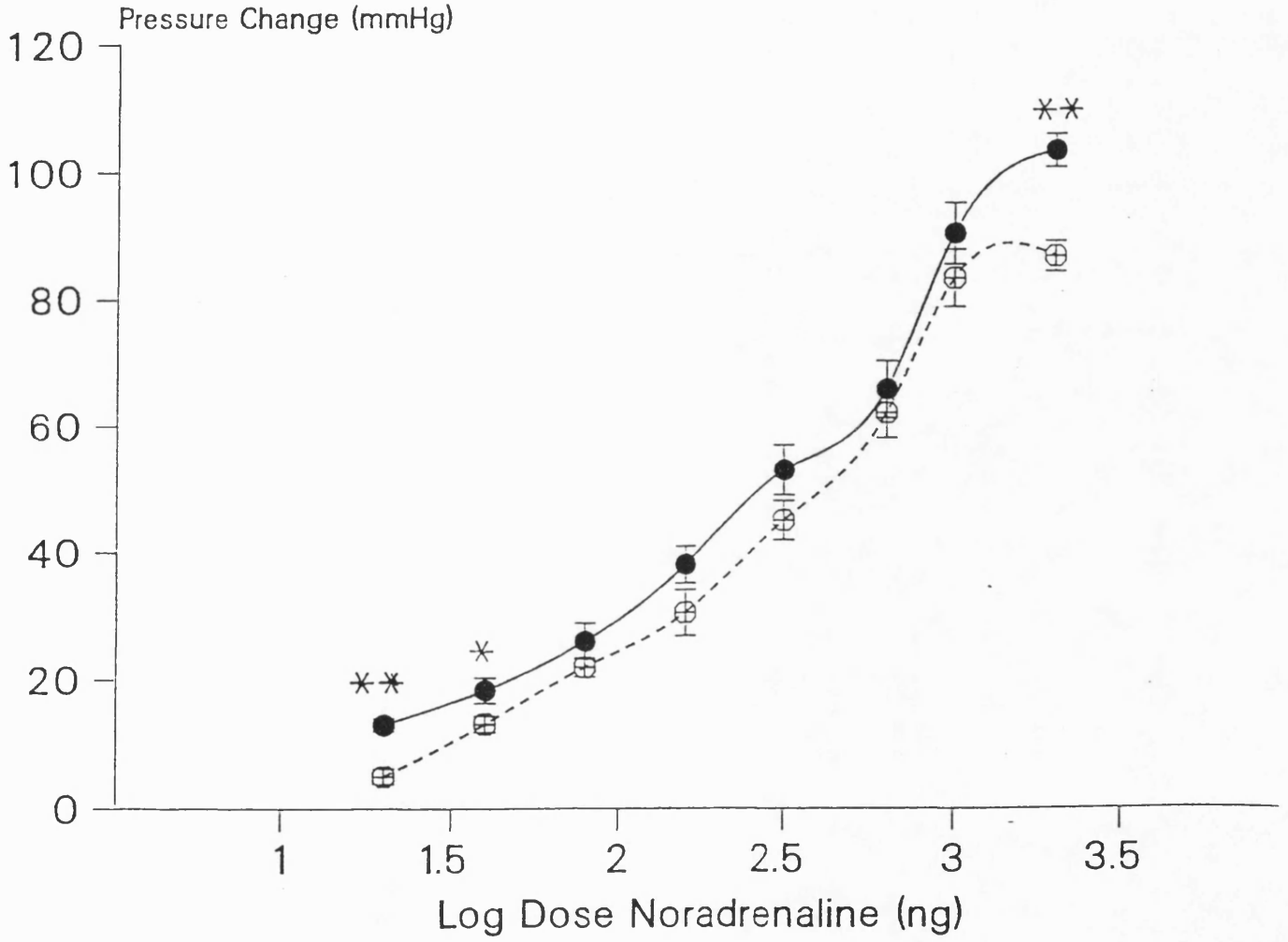


⊠----⊠ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=8) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 52

Graph showing the effect of 21 days pretreatment with the combination p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Wistar rat.



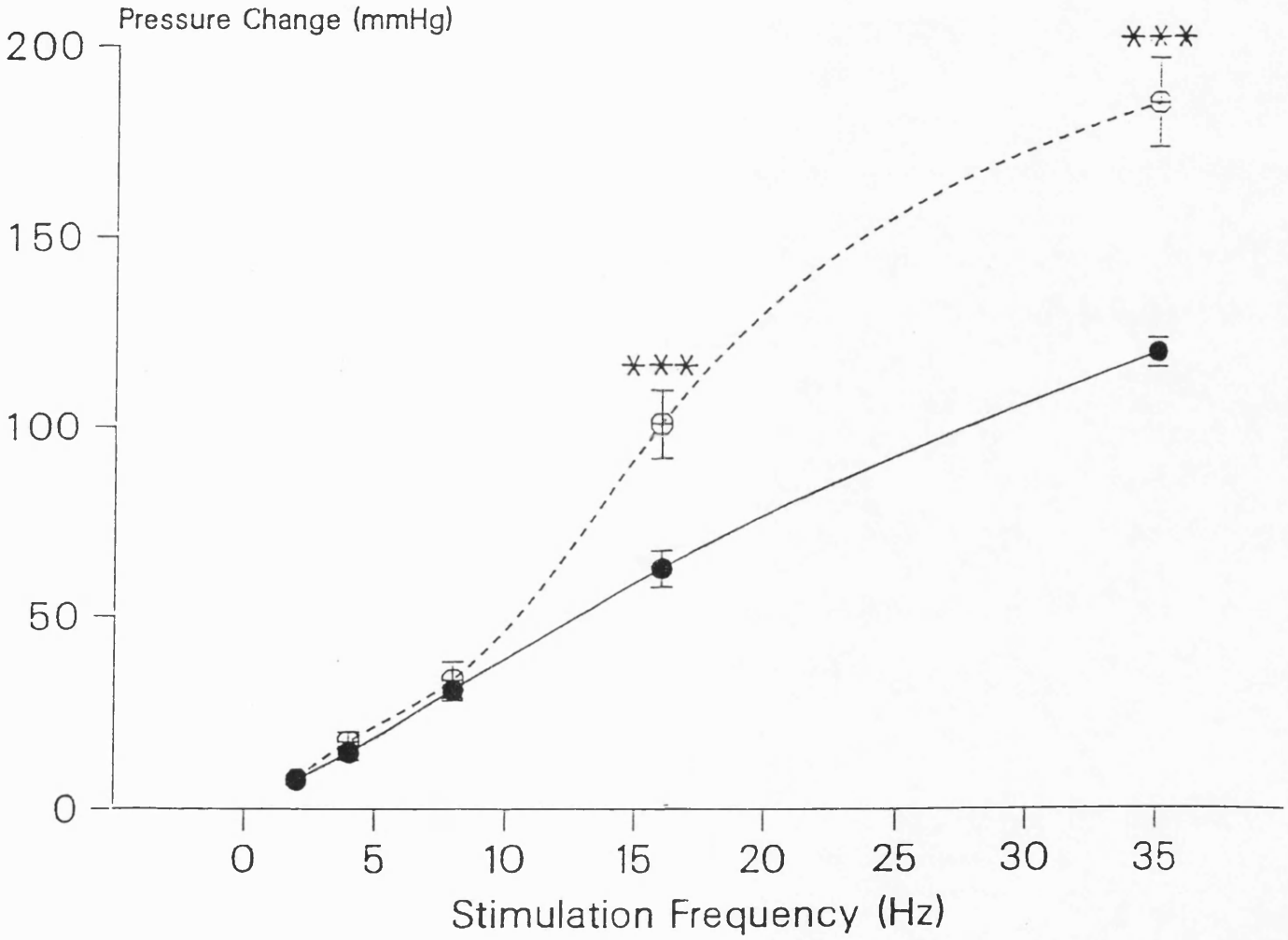
⊕----⊕ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=8) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=7) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 53

Graph showing the effect of 21 days pretreatment with the combination p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Wistar rat.



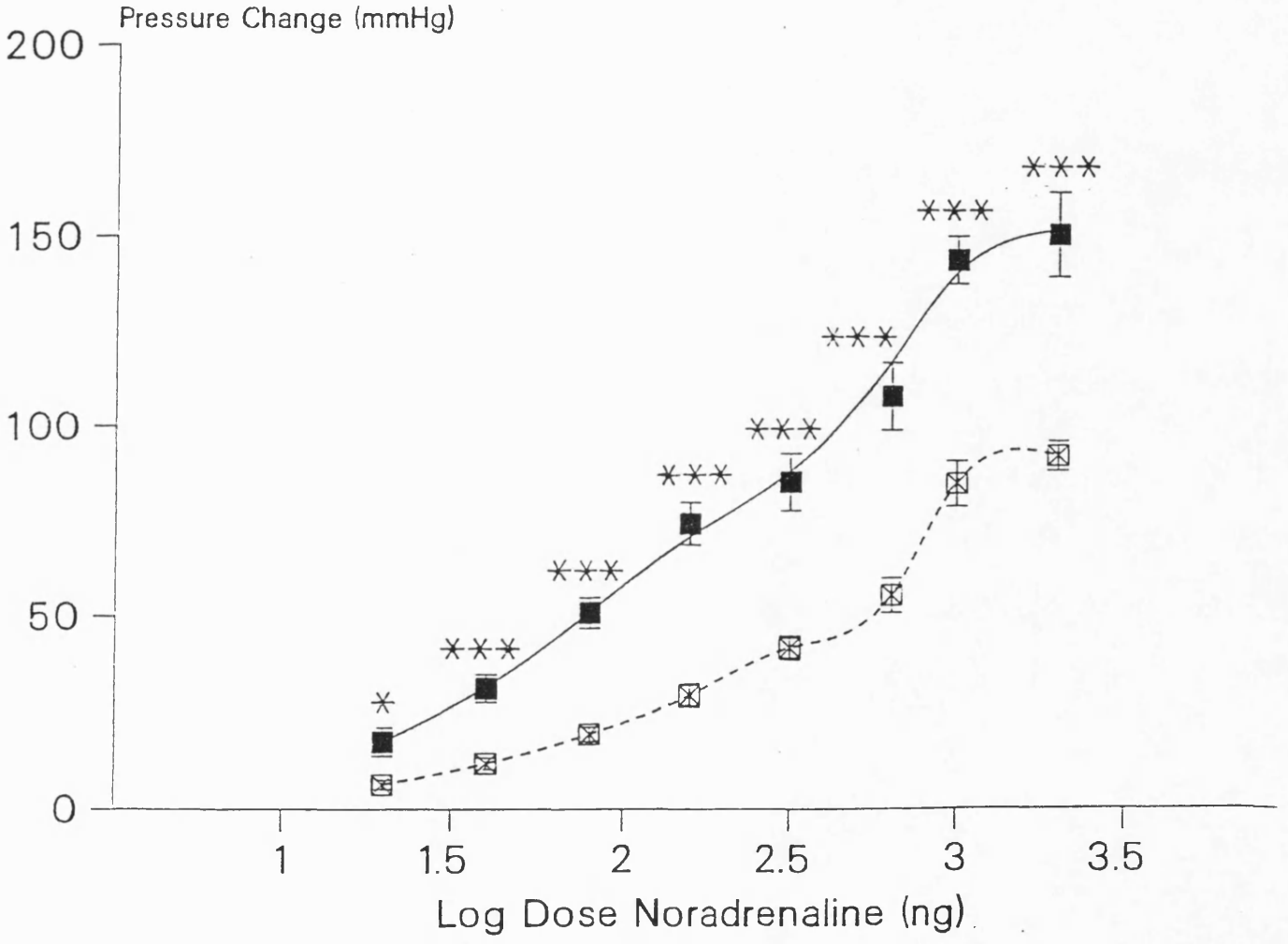
⊕----⊕ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=8) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=7) mean ±SE.

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE 54

Graph showing the effect of 7 days pretreatment with the combination p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Japanese Okamoto SHR.



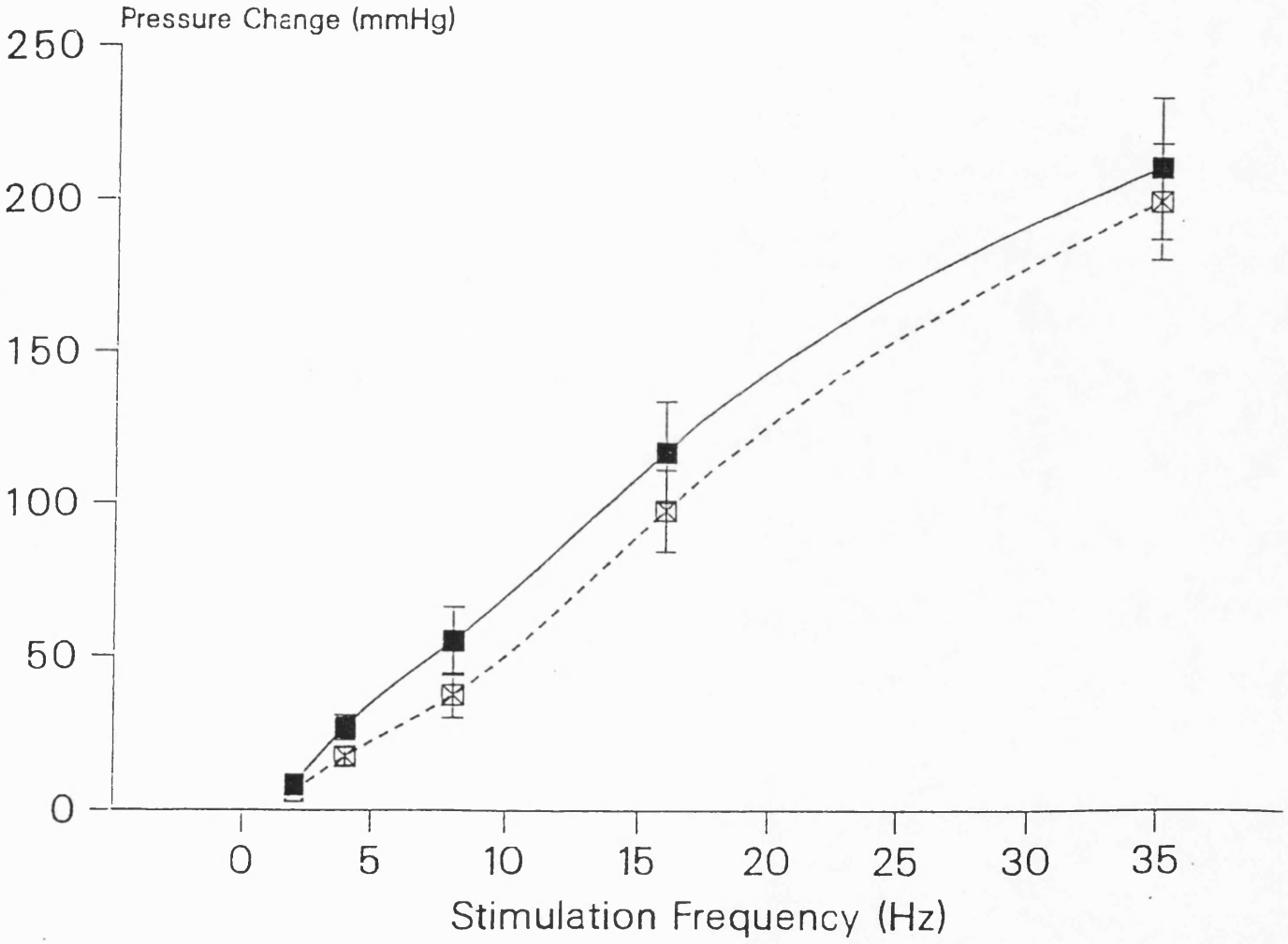
⊠----⊠ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=8) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE 55

Graph showing the effect of 7 days pretreatment with the combination p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Japanese Okamoto SHR.

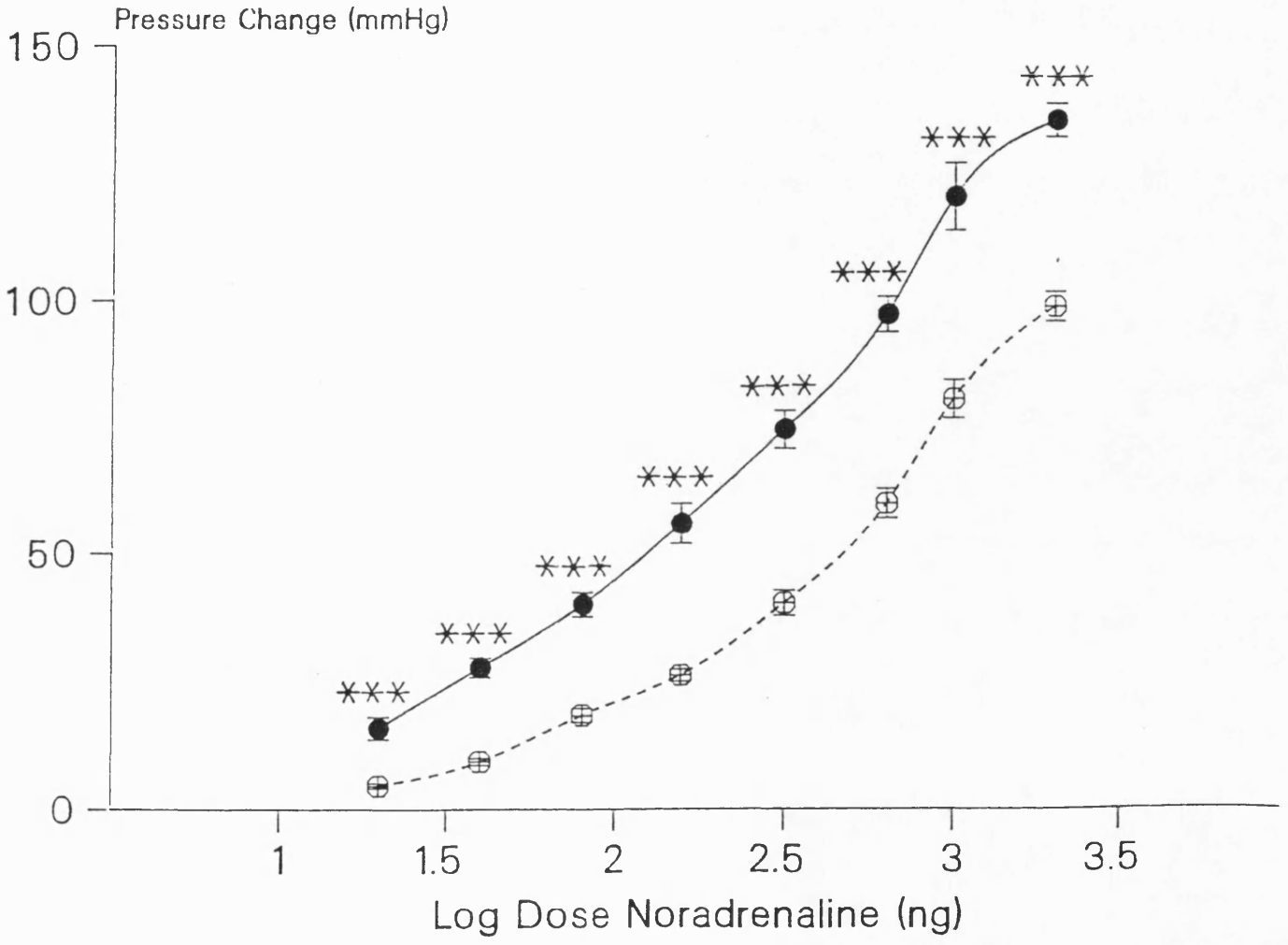


⊠----⊠ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=8) mean ±SE.

■——■ PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

FIGURE 56

Graph showing the effect of 21 days pretreatment with the combination p.o. on the mesenteric response to exogenous noradrenaline in the anaesthetised Japanese Okamoto SHR.



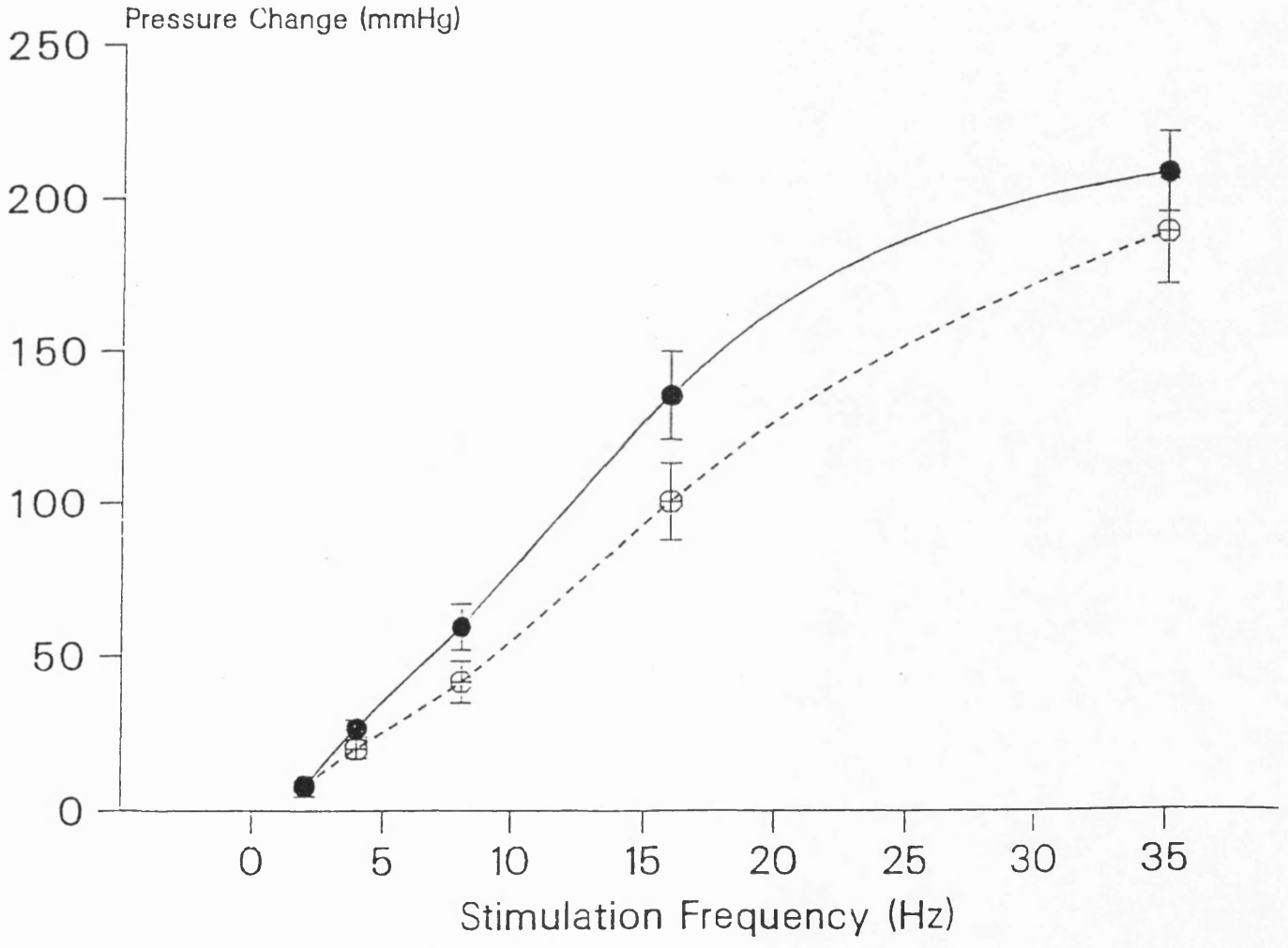
⊕-----⊕ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=9) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

* p<0·05 ** p<0·01 *** p<0.001

FIGURE 57

Graph showing the effect of 21 days pretreatment with the combination p.o. on the mesenteric response to periarterial electrical stimulation in the anaesthetised Japanese Okamoto SHR.



⊕----⊕ Combination (aten. 50mg/kg, nit. 3mg/Kg) p.o. (n=9) mean ±SE.

●——● PEG (5% 1ml/100g) p.o. (n=6) mean ±SE.

4.4 CONCLUSIONS.

4.4.1 Conclusions from the conscious rat studies.

The graphs depicting the blood pressure and heart rate responses of conscious animals (figures 44-47) are on pages 171-174.

Following treatment with the combination of atenolol and nitrendipine the blood pressure and heart rate of treated animals followed a similar pattern to that of similar animals treated with atenolol alone. There was no additive hypotensive effect observed with combination treatment. As the blood pressure of the spontaneously hypertensive rats was not reduced to normotensive levels it appears that the failure to observe an additive effect was not due to the hypotensive effect being maximal. There was evidence to suggest that, as previously described in atenolol-treated animals, there was a change in hypotensive action over the treatment period in the normotensive rats. As this possible mechanistic change was not apparent during treatment with nitrendipine alone, it is presumably due to a change in the tissue reponse to atenolol, although it is possible that this change is different from that observed with atenolol alone and due to some interaction between the two drugs.

The combination seemed to be reaching constant plasma levels in a similar way to that observed for the two drugs individually as the daily pre-/post-dose variation in blood pressure decreased in a similar fashion. This would appear to support the theoretical consideration that there would be no significant pharmacokinetic interaction between the two drugs. In addition to this, peak hypotensive effect was observed at two hours

after dosing, the same as with the two drugs given separately. This suggests that dosing with a combination did not significantly alter absorption times.

There was evidence to suggest that acute β-adrenoceptor blockade was not related to the hypotensive action of the combination, as β-mediated changes in heart rate did not closely "mirror" changes in blood pressure. Thus atenolol administered alone or in combination did not appear to be exerting its antihypertensive effect as a result of an acute β-blockade. The use of atenolol in combination with nitrendipine prevented the reflex tachycardia that was associated with nitrendipine given alone in normotensive animals. There was, however, no evidence of the combination interacting to produce any other effects on the heart rate.

4.4.2 Conclusions from the assessment of β-adrenoceptor blockade.

Following both 7 and 21 days pretreatment with the combination there was clear evidence of β-adrenoceptor blockade. The isoprenaline dose-response curves (figures 48 & 49) were shifted to the right after pretreatment with the combination. The addition of nitrendipine had no significant effect on the β-blockade, as the shift in the dose-response curves was similar to that obtained with atenolol alone. Interestingly, after 21 days pretreatment the isoprenaline shift was more parallel than that observed after atenolol treatment alone. It is possible that this is due to the chronic hypotensive action of the combination. This may reduce the relative importance of the β_2-mediated vascular component of the isoprenaline response, which may be, at least partly, responsible for the non-parallel shift. The increase in response to low doses of isoprenaline

observed after pretreatment with nitrendipine were not apparent when it was administered in the presence of atenolol. As this increase was thought to be due to a heightened reflex response to the drop in blood pressure (see section 3.4.2), which would be mediated by the action of catecholamines on the heart, it would seem likely that this was blocked by the action of atenolol on cardiac β_1-adrenoceptors.

4.4.3 Conclusions from the *in situ* blood perfused mesentery model.

The results from the investigation of adrenergic neurotransmission using the *in situ* blood perfused mesentery model were very interesting, they are described in section 4.3.4 and shown in figures 50-57.

The results obtained from animals treated with the combination are very hard to explain. The response to exogenous noradrenaline is similar to that obtained with nitrendipine alone, and there may be evidence of an additive effect with the combination. While there was only a limited decrease in response in normotensives the hypertensive response was reduced to a greater extent than that observed with nitrendipine or atenolol alone. Thus the reduction in response to exogenous noradrenaline in the hypertensive animals was large enough to be due to an additive effect of the drugs used in combination.

The responses of the animals to electrical stimulation was, however, much more complicated. When the two drugs were administered in isolation the overall effect of both was to reduce the response to electrical stimulation, and this was greater over time and in hypertensive animals. When the two drugs were used in combination this situation was reversed;

there was no change in response in hypertensive animals, a small decrease in response after 7 days in normotensives and a large increase in response after 21 days. Treatment with the combination appears to be producing some interaction to change the effect of each of the drugs on noradrenergic neurotransmission and this effect appears to change over time. When given alone nitrendipine appeared to have a postsynaptic effect which reduced the mesenteric response to both endogenous and exogenous noradrenaline. Atenolol in isolation had some prejunctional effect reducing the response primarily to endogenous noradrenaline. Despite this, when these two drugs were used together the response to endogenous noradrenaline appears to initially decrease and then increase greatly in normotensive animals, while remaining unchanged in hypertensive animals. The reasons behind these changes are not clear, but there are a number of possibilities. It seems that although the ability of the tissue to vasoconstrict in response to exogenous noradrenaline is reduced, its ability to respond to sympathetic stimulation is unchanged or increased. This suggests that the importance of noradrenaline as a transmitter at the sympathetic junction has been altered by the interaction of the combination. It is possible that the reduction of noradrenaline-induced vasoconstriction by atenolol and nitrendipine in combination is so profound that there is some physiological compensation via a non-adrenergic system. This may take the form of a change in one of the systems thought to regulate noradrenergic neurotransmission or possibly by the increase in the relative importance of a substance co-released with noradrenaline. The fact that the response to electrical stimulation was increased may indicate that while changes led to a "normalisation" of response in hypertensive animals it led to a "rebound" in response in normotensive animals.

What ever the complex changes produced by the combination are it must be remembered that the combination produced a hypotensive effect. The

changes observed may, however, be responsible for the fact that the combination did not have an additive hypotensive effect in the conscious animal.

4.4.5 Overall conclusions.

The combination reduced blood pressure in conscious rats this reduction was bigger in hypertensive animals. There was, however, no indication of any additive effect. The antihypertensive effect appeared to change over time, as blood pressure reached a minimum and then plateaued at a slightly higher level. Although this was not so apparent in hypertensive animals. The reflex tachycardia obtained in response to nitrendipine alone was antaganised by atenolol as it was not present whith the combination.

The combination had a larger effect on the response to noradrenaline than either drug in isolation, this may have been addative. This was seen in the results from the hypertensive rats only. Despite the fact that both drugs given alone produced a reduction in response to periarterial electrical stimulation; after combination treatment, an increase in some responses was noted. This did not appear to increase blood pressure, but may explain the absence of an additive hypotensive effect.

These conclusions are more fully discussed and compared with those from other treatment groups in chapter 5.

CHAPTER 5.

DISCUSSION

5.0 DISCUSSION.

5.1 Introduction.

This chapter discusses further the results and conclusions from all three treatment regimes. The discussion concentrates on the investigation of adrenergic neurotransmission, in an attempt to explain the drug effects on blood pressure, and to suggest possible mechanisms of drug action and interaction. This is followed by suggestions of further work that would be required to firmly establish these theories. To facilitate the comparison of the treatment groups the discussion is preceeded by a tabular summary of the results obtained.

5.2 Summary of results.

Normotensive Beagles
(21 days atenolol)

	Exogenous Noradrenaline	Electrical Stimulation
Mesenteric Pressure response	=	=↓
Mesenteric Flow response	=	=
Mesenteric Resistance response	=	↓↓

Normotensive Wistar Rats

	ATENOLOL		NITRENDIPINE		COMBINATION	
	7 days	21 days	7 days	21 days	7 days	21 days
Conscious Blood pressure	↓↓	↓↓	↓	↓	↓↓	↓↓
Conscious Heart rate	↓	↓	↑	↑	↓	↓
Isoprenaline response	↓↓	↓↓	↑	↑	↓↓	↓↓
Noradrenaline response	=	=	=	=	=	=↓
Electrical stimulation	=	↓	=↓	↓	=↓	↑↑

Spontaneously Hypertensive Rats (SHR)

	ATENOLOL		NITRENDIPINE		COMBINATION	
	7 days	21 days	7 days	21 days	7 days	21 days
Conscious Blood pressure	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Conscious Heart rate	↓↓	↓↓	=	=	↓↓	↓↓
Noradrenaline response	=	↓	↓	↓↓	↓↓↓	↓↓↓
Electrical stimulation	=	↓↓↓	↓↓	↓↓↓	=	=

5.3 Discussion of the conscious rat studies.

Overall, the results obtained from the investigation of blood pressure and heart rate in conscious animals were interesting. They revealed, that atenolol appeared to have an antihypertensive effect that was distinct from β-adrenoceptor blockade, and possibly that this changed with chronic administration.

Nitrendipine reduced blood pressure in conscious rats, but this was less than that observed with the other treatments and was associated with a reflex tachycardia in normotensive animals. This was consistent with the theory that the dihydropyridine calcium antagonists are only really effective when peripheral resistance is initially raised, such as in the SHR.

The expected additive effect of the combination treatment was not apparent, and there was evidence that there may also be mechanistic changes over time with this group. This may, or may not, be as a result of changes in the mechanism of atenolol's antihypertensive action. Although the prevention of the nitrendipine induced tachycardia did not cause a greater reduction in blood pressure, it is a positive interaction, as it would alleviate the clinically reported side effects that result from increased heart rate.

5.4 Discussion of the assessment of β-adrenoceptor blockade.

Atenolol produced β-blockade when administered alone and in combination. This was present after 7 days pretreatment and was still apparent after 21 days. There was no evidence of nitrendipine causing any

β-blockade; it appeared, however, to increase the response to isoprenaline, presumably as a reflex response to vasodilation.

One of the considerations to come from this work was, that although the anaesthetised rat preparation was able to show whether β-adrenoceptor blockade was present, the results were somewhat confused by the complexity of the situation. There is an argument for using an isolated tissue preparation to investigate β-blockade, as such a preparation would be simpler to interpret. This is discussed further in the section on future work.

5.5 Discussion of the investigation of noradrenergic neurotransmission.

This section of the work has produced interesting results that have led to a number of conclusions.

Atenolol appears to be acting presynaptically, possibly to reduce the release of noradrenaline. This has been shown in normotensive and hypertensive rats and in normotensive dogs. Interestingly, the size and time-course of this effect suggest that it may be important in the antihypertensive effect of atenolol observed clinically. As the presynaptic effect of atenolol is not apparent after 7 days treatment, but is established by 21 days, this change may explain the "plateau" effect seen in the blood pressure study.

Nitrendipine appears to be inhibiting the effects of both endogenous and exogenous noradrenaline, presumably via the blockade of Ca^{++}-channels associated with the excitation-contraction coupling of the vascular smooth muscle.

The combination would, perhaps, be expected to display both these previously described properties; as a result of each drug acting separately. On initial examination this appears not to be so.

The combination undoubtedly produced a marked inhibition of exogenous noradrenaline, but electrical stimulation produced some unexpected results. In order to try and explain these results, it is perhaps prudent to look at the mechanisms by which the two drugs are acting when given separately, in more detail.

The main antihypertensive effect of nitrendipine, is a result of its blockade of the Ca^{++}-dependent excitation-contraction coupling of smooth muscle. As endogenous noradrenaline acts on predominantly post-junctional α_1-adrenoceptors in the mesentery, it follows that nitrendipine can inhibit the Ca^{++}-dependent vasoconstriction associated with these receptors. The α_1-adrenoceptors are thought to cause either Ca^{++} release from the sarcoplasmic reticulum, or Ca^{++} entry through receptor-gated channels. Nitrendipine would not be expected to antagonise either of these α_1-adrenoceptor induced Ca^{++} fluxes. It would, however, antagonise the Ca^{++} flux through the voltage-gated channels that these initial changes would cause (Van Breemen *et al* 1980, 1986). This is how nitrendipine is likely to inhibit the excitation-contraction coupling procedure in the mesentery. The results obtained from the *in situ* mesentery model support this conclusion. They do not, however, eliminate other possible actions.

The investigation does not preclude nitrendipine having a diuretic and/or natriuretic hypotensive effect. Although some workers suggest that these aspects of nitrendipine's antihypertensive effect are important (Garthoff *et al* 1983, Hall & Hungerford 1984), they would not account for the changes observed in mesenteric noradrenergic neurotransmission. This does not, however, imply that they do not play a part in the overall

antihypertensive action of nitrendipine. The well documented effects of nitrendipine on the structural abnormalities (eg. ventricular hypertrophy) of hypertension may also play a part in its overall hypotensive effect; but there was no histopathological study done in this investigation to confirm or refute this possibility.

The antihypertensive action of atenolol is a matter of some debate. This study would seem to confirm that the blockade of β_1-adrenoceptors plays no direct role in its chronic effects, and suggests that it acts via some presynaptic interaction. The exact nature of this presynaptic effect is not certain. There is a possibility that some β-blockers act by blocking the presynaptic β_2-adrenoceptors, preventing the facilitation of noradrenaline release by adrenaline (Frishman & Silverman 1984). However, it would seem unlikely that this is the presynaptic mode by which atenolol acts, as it is β_1-adrenoceptor specific. Also, results suggest that the time course of its hypotensive effect is distinct from that of β-adrenoceptor blockade.

There is also the possibility that atenolol may cause depletion of noradrenaline at the sympathetic terminal via interactions with its turnover (Alexandre & Chevillard 1980) and/or uptake (Street & Walsh 1985). This would cause a reduction in the stimulus-induced release of noradrenaline as seen in the investigation. However, these actions appear to require a higher degree of lipid solubility and membrane stabilising properties than possessed by atenolol.

The theory that atenolol may act presynaptically to inhibit the facilitatory effect of locally released angiotensin II (Jackson & Campbell 1980b) can not be discounted by this work. It seems likely that chronic

atenolol may act via locally produced prostaglandins to achieve this inhibition. Prostaglandins E_2 and I_2 have been shown to be able to inhibit the angiotensin II facilitation of noradrenergic neurotransmission (Jackson & Campbell 1980a, Mizuno *et al* 1988) and it has been established that they may be produced locally (Westfall 1977, Gryglewski *et al* 1988). Recently it has been shown that after chronic administration β-blockers may increase the prostaglandin (particularly PG E_2) levels in vascular tissue and thereby inhibit the facilitatory role of angiotensin II (Jackson & Campbell 1981, Daniell *et al* 1988). This theory is supported by the clinical observation that indomethacin, a prostaglandin synthesis inhibitor, can reduce the antihypertensive action of β-blockade (Durao 1977, Salvetti *et al* 1984). Although this mechanism of action was not investigated, the time course of the presynaptic effect of atenolol observed in this work is similar to that described for the prostaglandin/angiotensin II interaction.

Other possible antihypertensive actions of atenolol cannot be discounted by this work. Plasma renin activity was not measured, and some workers suggest that β-adrenoceptor blockers may act by decreasing this activity (Bühler *et al* 1975). While there is undoubtedly evidence that some β-adrenoceptor blockers can reduce renin release via interactions with renal β_2-adrenoceptors, this does not seem to be correlated with their antihypertensive ability. Additionally, β_1-bloking agents, such as atenolol, appear to have no effect on plasma renin activity (Antonaccio *et al* 1986), and the hypotensive effect observed in this work does not appear to be related to blockade of β-adrenoceptors. Also, the apparent presynaptic effect of atenolol revealed in this study could not be easily explained in terms of altered renin activity.

The idea that β-blockers may produce their antihypertensive effect by interaction with the baroreceptor reflex (Pickering *et al* 1972, Scott 1981)

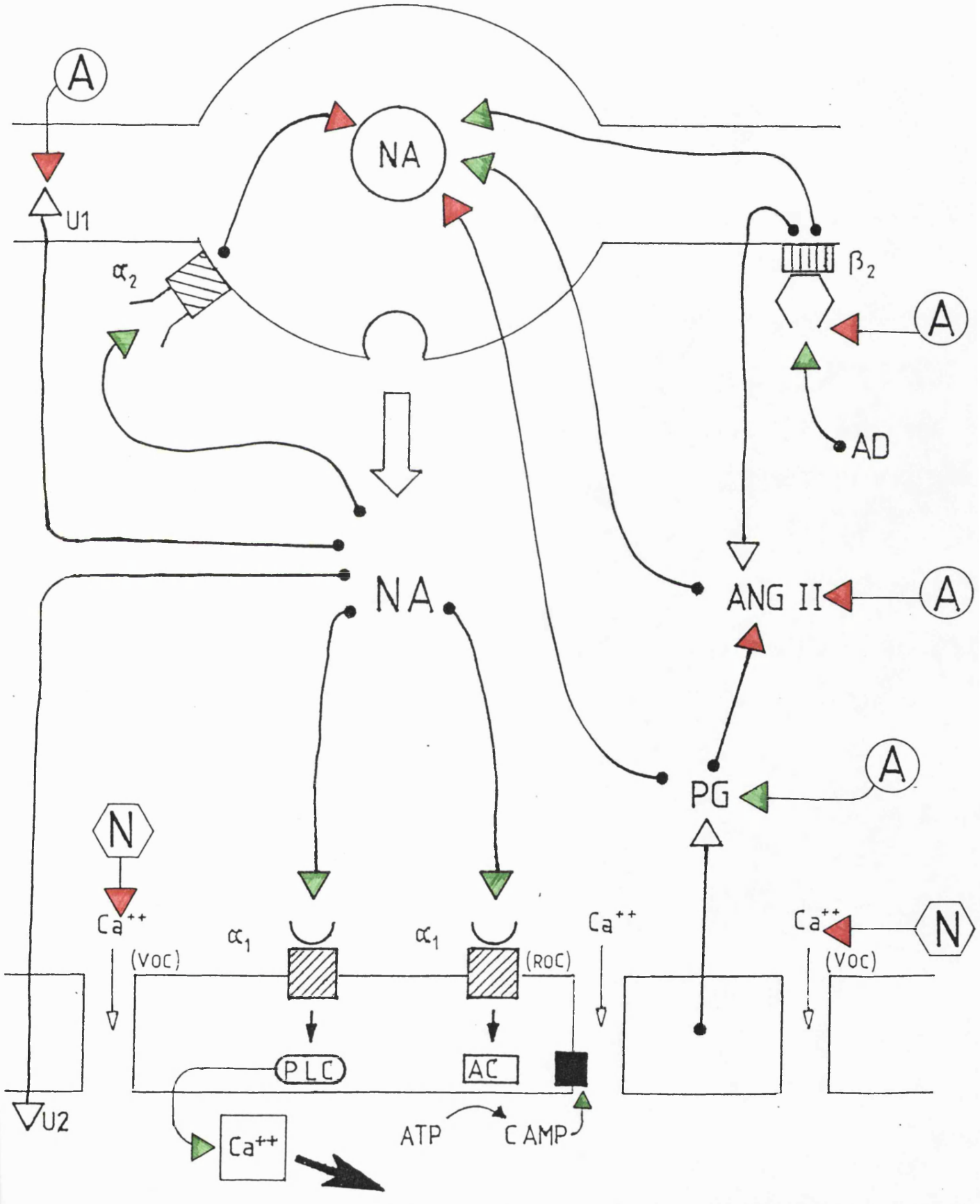
can not be discounted by the present investigation. It would seem, however, that while such an effect may alter the sympathetic response to changes in blood pressure, it would not be expected to reduce stimulus induced transmitter release. Therefore, although it may play a part in the overall antihypertensive effect, it would appear not to be directly involved in the changes in neurotransmission observed in this work.

As no histopathological investigation was undertaken, the effect of atenolol on arterial hypertrophy and hyperplasia cannot be determined. While it is possible that these conditions were "reversed" by chronic atenolol treatment, it is unlikely that these effects would be entirely responsible for its hypotensive action. It is feasible that by changing the ratio between the vessel wall thickness and lumen size a drug may appear to be exerting a presynaptic effect. As the sympathetic nerve fibres are in the medial layer of the wall, and exogenously administered drugs have to diffuse through from the lumen, changing the wall thickness may alter the relative "efficiency" of these processes. If this was the case, response to sympathetic stimulation may be reduced because of a reduction in vessel wall "strength", while the effect of exogenous drugs may appear to be unchanged or increased, as drug diffusion across the thinner wall would be easier. This would fit with the observed results and the time course of the atenolol effect. It is, however, an unlikely explanation of the observed effects. Such structural changes occur because of a reduction in "load" on the vessel, in terms of pressure (Laplace's law); therefore all drugs which reduced pressure would be expected to have the same effect. As all three treatment regimes reduced pressure, and nitrendipine is known to reverse these structural changes, it would be expected that all the treatments would appear to have this same presynaptic effect. This was not the case, so it would seem likely that although

structural changes may play a part, they are not responsible for the presynaptic effect observed with atenolol.

From this discussion, it would seem that nitrendipine is affecting noradrenergic excitation-contraction coupling via its action on voltage operated calcium channels, while atenolol appears to be having some presynaptic effect, which may reflect changes in uptake and storage of noradrenaline and/or alterations of the prostaglandin/angiotensin II regulation of noradrenaline release. These effects are shown in the diagram of noradrenergic neurotransmission on the following page (figure 59).

FIGURE 59: A schematic diagram of noradrenergic neurotransmission showing the effects of nitrendipine and atenolol.



LEGEND:

NA = Noradrenaline

AD = Adrenaline

ANG II = Angiotensin II

PG = Prostaglandin

PLC = Phospholipid-cholesterol complex

AC = Adenylate cyclase

ATP = Adenosine triphosphate

CAMP = Cyclic adenosine monophosphate

ROC = Receptor operated calcium channel

VOC = Voltage operated calcium channel

U1 = Uptake 1

U2 = Uptake 2

α_1 = α_1-adrenoceptor

α_2 = α_2-adrenoceptor

β_2 = β_2-adrenoceptor

= Nitrendipine

= Atenolol

Red arrow-head = Inhibitory effect.

Green arrow-head = Facilitatory effect.

There appears to be no obvious interaction between atenolol and nitrendipine that could explain the effects of the combination treatment on neurotransmission. Further investigation revealed, that while there is no apparent link between prostaglandins and the action of nitrendipine, the actions of prostaglandins are Ca^{++}-dependent. Therefore, it is possible that nitrendipine may interact with atenolol through its prostaglandin-mediated actions and that this may help to explain the confusing results obtained with the combination.

The phospholipase-dependent release of arachidonic acid from the membrane is the rate-limiting step for prostaglandin biosynthesis. The activity of these phospholipases is Ca^{++}-dependent; Van de Velde and co-workers (1986) showed that the calcium antagonist nifedipine could suppress this synthesis by around 50%. Calcium antagonists are not able to inhibit this biosynthesis fully as intracellular Ca^{++} accounts for about half of the phospholipase activation. It would seem that although nitrendipine may reduce the atenolol mediated prostaglandin action, it would be unable to prevent it.

Interestingly, it has been shown that the inhibitory effect of prostaglandins on noradrenergic neurotransmission may be reversed by increasing the availability of Ca^{++} (Hedqvist 1970). It has been suggested that prostaglandins may act by indirectly inhibiting the Ca^{++} flux in the nerve, which is responsible for the focal extrusion of noradrenaline into the synaptic cleft, and so reduce transmitter release (Stjärne 1973, Hedqvist 1976). If this is the case, it is possible that nitrendipine may also inhibit this flux and add to the inhibitory effect of the prostaglandins. Should this occur, both atenolol and nitrendipine would act to reduce noradrenaline release drastically.

This possible interaction between atenolol and nitrendipine would undoubtedly result in a drastic reduction in the release of noradrenaline, but this alone does not explain the results obtained with the combination treatment.

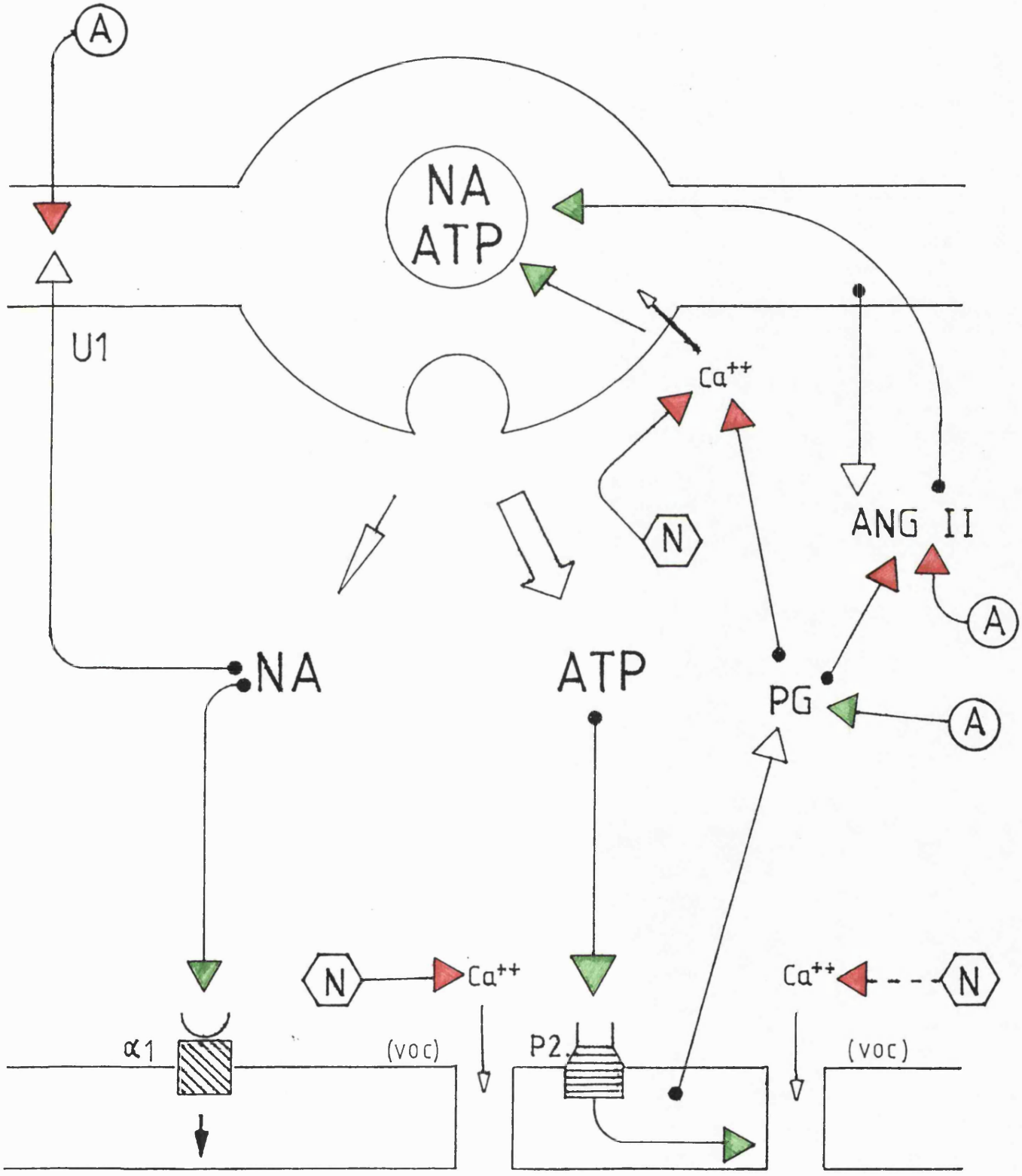
As long ago as the mid 1960's it was recognised that the effects of sympathetic neurotransmission in the mesentery were not completely reproduced by exogenous noradrenaline (Mc Gregor 1965). It is now widely thought that other biologically active compounds may coexist, and be co-released with noradrenaline in the sympathetic nervous system (Burnstock 1986, Campbell 1987). While the physiological role of such co-transmitters has not been fully established, their effects can clearly be seen when the main transmitter (noradrenaline) has been depleted (Campbell 1987).
There is evidence to suggest that co-transmission does occur in the mesenteric vascular bed, it has been suggested that adenosine triphosphate (ATP) (Burnstock 1985, 1986, 1988) and/or neuropeptide Y (NPY) (Campbell 1987, Lundberg & Hökfelt 1985) are co-released with noradrenaline. Both these compounds are known to cause vasoconstriction, and either, or both of them may act to produce such an effect when the action of noradrenaline has been diminished.

It is possible that chronic treatment with the combination produces such a profound reduction in the release of noradrenaline via the interaction with the prostaglandin mediated system, out-lined above, that a co-transmitter is "unmasked". This would explain the absence of a decreased response to periarterial electrical stimulation in animals treated with the combination. The difference between the normotensive and hypertensive animals is, however, still not easily explained. It is possible that the co-transmitter is relatively less important in the SHR where there is greater noradrenaline release. Alternatively, the co-

transmitter may play a larger part in the "normal" untreated SHR, possibly contributing to the cause and/or maintenance of the hypertension, while in the normotensive animal its role is "swamped" by noradrenaline. Thus, when it is "unmasked" there is a rebound increased response in the normotensive. Interestingly, the release of co-transmitters occurs preferentially at higher stimulation frequencies (Burnstock 1986), and the increase in mesenteric response to electrical stimulation observed following combination treatment, occurred at the higher frequencies. This lends further support to the theory that co-transmission may be involved in the unexpected findings observed with the combination.

In order to illustrate this theory, the schematic diagram on the following page shows noradrenergic neurotransmission following chronic treatment with the combination (Figure 60). In this simplified example the putative co-transmitter ATP alone is included for clarity. The ATP is thought to interact with post-synaptic P_2-purinoceptors to cause vasoconstriction via electromechanical coupling to voltage dependent Ca^{++}-channels. Although these channels are blocked by nitrendipine, the interaction with the P_2-purinoceptor may change the state of the channel from "inactive" to "open", and so displace the nitrendipine allowing the excitation-contraction coupling to precede. To simplify the diagram, the presynaptic P_1-purinoceptor, which inhibits the release of ATP and noradrenaline, is not included.

FIGURE 60: A schematic diagram of noradrenergic neurotransmission showing co-transmission and the effects of combination.



LEGEND:

NA = Noradrenaline
ATP = Adenosine triphosphate
ANG II = Angiotensin II
PG = Prostaglandin
VOC = Voltage operated calcium channel
U1 = Uptake 1
α_1 = α_1-adrenoceptor
P_2 = P_2-purinoceptor

= Nitrendipine

= Atenolol

Red arrow-head = Inhibitory effect.

Green arrow-head = Facilitatory effect.

It appears that following chronic administration, atenolol and the combination effect noradrenergic neurotransmission by interacting with a transmitter release "control" mechanism. Evidence suggests that locally produced prostaglandins and angiotensin II interact with the sympathetic nerve varicosities, perhaps to "fine tune" transmitter release. If this is the case, it is interesting to speculate on the role of this system in the control of blood pressure. It is possible that such a system may be involved with the cause and/or maintenance of hypertension. Also, as this local mediation would be unlikely to remain "intact" in isolated, Krebs perfused systems, it may, at least partially, explain the differences in sensitivity to nerve stimulation observed between Krebs and blood perfused mesentery preparations.

While the theories outlined above are supported by the experimental evidence of other authors, and are consistent with the results obtained in this work, they are not all directly shown by the present investigation.

This investigation has produced some interesting results, and has allowed a number of conclusions to be drawn. It has also raised many questions, a great deal of further work could be done to answer these questions, and this is discussed in the following section.

5.6 Suggestions for further work.

Perhaps the most obvious direction for further work is to "complete" the initial work scheme in both rats and dogs. The diagram (Figure 1) on page 4, shows a flow chart of the work undertaken so far. It would be interesting to "fill in" the missing places in the flow chart for all the species, and perhaps include hypertensive dogs. This would complete the picture in terms of the present work, and allow further investigation of the trends observed in rats in another species.

The investigation of ^{3}H-noradrenaline overflow could be extended to include all treatment groups and hypertensive animals. It would be particularly revealing to investigate the release of noradrenaline following treatment with the combination. Also, differences in release, and the treatment affects, in the hypertensive animal would be interesting. As the presynaptic effects observed after treatment with both atenolol and the combination appear to occur after chronic administration, further investigation of the time course of the effect would seem appropriate. Changes in the mesenteric response and in the stimulus-induced release of transmitter could be investigated after a single acute dose of drug, perhaps 2 and 24 hours after such a dose. Also the length of time of chronic dosing could be varied from 2 to 21 days and perhaps longer. Investigation of adrenergic neurotransmission after such a dosing scheme would allow the time course of any chronic presynaptic effect to be charted. As there seems to be a change in the hypotensive mode of action of both atenolol and the combination between 7 and 21 days it would be informative to find out when this actually happened, and if the effect continued over a longer period.

The information obtained about transmitter release by using ^{3}H-noradrenaline is useful, but it does not distinguish between labelled noradrenaline or labelled inactive metabolites. As it is possible that drug treatment may affect the metabolism of transmitter, another method of measuring catecholamine release (perhaps HPLC) could possibly be run in parallel with such studies.

The work undertaken so far has not provided any evidence of the effect of drug treatment on the uptake of noradrenaline. As it has been suggested that atenolol may effect this process, an investigation of the uptake of ^{3}H-noradrenaline into various tissues could be undertaken. This could be combined with a study of the effect of acute blockade of uptake 1 (with cocaine) on the effect of atenolol on neurotransmission in the mesentery.

The involvement of locally released angiotensin II and prostaglandins with the actions of the drugs could be investigated by examining the effects of acute doses of indomethacin (a prostaglandin synthesis inhibitor) and saralasin (an angiotensin II antagonist) alone and in combination in animals chronically pretreated with the three treatment regimes under investigation. Exogenous prostaglandins and angiotensin II could also be used together with their respective inhibitors to examine whether the prostaglandins exert their effect via inhibition of the angiotensin II potentiation of transmitter release, or directly. If locally released prostaglandins are involved in the control of noradrenaline release and perhaps blood pressure in the mesentery, it would be interesting to examine the effects of chronic administration of indomethacin and sulindac. Indomethacin inhibits all prostaglandin synthesis, while sulindac has been reported as inhibiting only systemic prostaglandin synthesis (Ciabattoni *et al* 1980). These drugs may therefore

help to establish the importance of vascular as opposed to renal prostaglandins in the control of blood pressure.

The importance of the release of co-transmitters in the response to the combination could be investigated further, by using the appropriate antagonists to try and reverse the increased response to nerve stimulation observed after combination treatment. As the co-transmitter(s) involved are not known this would be a matter of some trial and error. As there seems to be evidence that ATP is an important co-transmitter, perhaps the P_2-purinoceptor antagonist arylazido aminopropionyl-ATP ($ANAPP_3$) would be an appropriate first choice. If this is found to reduce the increased response to nerve stimulation following chronic treatment with the combination, it would be interesting to investigate whether stimulation of P_2-purinoceptors would allow Ca^{++} entry through voltage operated channels in the presence of nitrendipine. This could be examined initially by observing the effects of exogenous ATP on the response to endogenous and exogenous noradrenaline following acute and chronic nitrendipine treatment. This could then be investigated in more detail using patch-clamping techniques to examine the actual fluxes of ions. If it was found that the combination was producing such a profound decrease in the release of noradrenaline that a co-transmitter was "unmasked", it would be interesting to investigate the effects of lower doses of atenolol, nitrendipine and the combination. This may help to establish whether the hypotensive effects were additive when the reduction of noradrenaline release was less profound.

In addition to the previously discussed further work, the investigation of β-adrenoceptor blockade using an anaesthetised animal preparation was questioned earlier in the study. It would, perhaps, be prudent to investigate the explanation of the anomalies proposed earlier by

using an isolated heart preparation. This preparation would be free of the complications due to "whole animal" effects, and could be run in parallel with the anaesthetised animal method using isolated hearts from pretreated animals. However, isolated preparations contravene the aim of undertaking work *in-situ*; a pithed rat preparation may, perhaps, be preferable.

The ultimate aim of these further investigations would be to fulfil the initial aims of the study and find out how these drugs were producing their antihypertensive effect following chronic administration. The evaluation of the interactions observed between these two drugs following their combined administration, may provide information that would allow their clinical use in combination to be more effective and safer.

In addition to this, throughout the work the results obtained from the various methods used have suggested that *in situ* blood perfused preparations are sensitive and consistent, but can produce complicated results. It appears that while isolated tissues produce results that are more easy to interpret, there may be considerable differences between such situations and the physiological norm. It follows therefore, that to provide results that are more physiologically applicable, preparations that provide conditions close to "life" should be used. To take this to its logical extreme, it would be advisable to develop, and use telemetric systems for measuring blood pressure and flow to various vascular beds. Such systems could then be used to examine the effects of treatment regimes in free living, conscious animals with relatively little interference by the investigator. While this sort of system would provide complex data that would be of little use on the molecular level of drug/receptor interaction, it would be invaluable for evaluating the effects of drugs on the "normal" physiology of animals, and may provide high quality data that could be more safely extrapolated to the situation in man.

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APPENDICES.

APPENDIX 1.

Program used to calculate change in resistance.

```
 10      REM    PROG TO CALC CHANGE IN RESISTANCE
 20      D=0,   REM D = dog number
 30      B=0,   REM B = mean control BP
 40      F=0,   REM F = control Flow
 50      R=0,   REM R = control Resistance
 60      D$=0,  REM D$ = dose
 70      B1=0,  REM B1 = mean BP response
 80      F1=0,  REM F1 = Flow response
 90      R1=0,  REM R1 = Resistance response
100      F2=0,  REM F2 = change in Flow
110      R2=0,  REM R2 = change in Resistance
120      PRINT  "Input Dog Number  (F to finish)"
130      INPUT D
140      IF D = F THEN 340
150      PRINT  "Dog Number ......" D
160      PRINT  "Dose ?"
170      INPUT D$
180      IF D$ = No  GOTO 120
190      PRINT  "Input control Blood Pressure"
200      INPUT B
210      PRINT  "Input control Flow"
220      INPUT F
230      PRINT  "Input control response Blood Pressure"
240      INPUT B1
```

```
250       PRINT  "Input response Flow"
260       INPUT F1
270       R = ( B * 79980 ) / F
280       R1 = ( B1 * 79980 ) / F1
290       R2 = R1 - R
300       F2 = F1 - F
310       PRINT  "Dose  ="  D$
320       PRINT  "Change in minute Flow  ="  F2,
                 "Change in Resistance  ="  R2
330       GOTO  150
340       PRINT  "End of Run"
350       END
```

APPENDIX 2.

Summary of base-line conditions in the mesenteric vasculature.

1. Wistar Rats - 7 days treatment.

Treatment	$_{s}$BP(mmHg)	$_{\bar{m}}$BP(mmHg)	$_{m}$R (Kdyne/s/cm^{5})
PEG	121·0±6·9	130·2±12·2	5238·7±488·1
Atenolol	109·6±3·7	155·7±12·1	6229·2±483·4
Nitrendipine	88·8±4·9	119·7±9·8	4787·4±393·2
Combination	79·3±5·0	96·4±6·1	3856·2±242·8

2. Wistar Rats - 21 days treatment.

Treatment	$_{s}$BP(mmHg)	$_{\bar{m}}$BP(mmHg)	$_{m}$R (Kdyne/s/cm^{5})
PEG	85·4±4·1	114·3±6·7	4569·8±269·0
Atenolol	110·0±6·1	128·4±4·3	5135·9±170·0
Nitrendipine	130·2±5·3	127·7±5·6	5106·7±222·9
Combination	99·0±5·8	127·5±7·3	5097·2±293·4

3. Japanese Okamoto SHR's - 7 days treatment.

Treatment	$_{s}$BP (mmHg)	$_{m}$BP (mmHg)	$_{m}$R (Kdyne/s/cm^{5})
PEG	128·3±5·8	117·2±7·4	4685·5±294·2
Atenolol	154·7±5·7	160·6±12·3	6421·3±491·0
Nitrendipine	145·3±4·8	152·5±7·8	6098·5±311·8
Combination	116·5±6·1	127·0±8·4	5078·7±337·9

4. Japanese Okamoto SHR's - 21 days treatment.

Treatment	$_{s}$BP (mmHg)	$_{m}$BP (mmHg)	$_{m}$R (Kdyne/s/cm^{5})
PEG	135·7±10·7	126·7±6·1	5065·±244·5
Atenolol	97·7±7·3	99·7±3·8	3985·7±150·1
Nitrendipine	96·7±7·0	95·3±1·7	3812·4±66·4
Combination	104·7±3·4	114·2±3·3	4567·7±132·2

LEGEND.

$_{s}$BP = Systemic Mean Blood Pressure (±SE)
$_{m}$BP = Mesenteric Mean Blood Pressure (±SE)
$_{m}$R = Mesenteric Resistance (±SE)

5. Untreated Normotensive Male Beagles.

Dog No.	H. R.	$_{sa}$BP	$_{m}$BP	$_{\bar{m}}$BP	F	R.
980	171·6	140·3	135/108	117	285	32·8
839	156·7	109·0	123/108	107	104	83·1
1081	177·5	119·8	124/95	105	60	140·0
1488	167·8	142·8	158/124	135	92	116·9
1400	152·0	97·7	114/84	94	146	51·6
1486	199·0	144·3	160/130	140	154	72·6
Mean	**170·8**	**125·7**	**136/107**	**116**	**140**	**82·8**
± SE	±6·8	±8·1	7·9/7·2	±7·4	±32·3	±16·3

6. Normotensive Male Beagles Treated for 21 days with Atenolol (7·5mg/Kg).

Dog No.	H. R.	$_{sa}$BP	$_{m}$BP	$_{\bar{m}}$BP	F	R.
1202	153·0	144·0	155/125	135	135	70·0
1369	150·5	146·5	162/128	139	150	74·1
913	150·8	110·8	120/95	103	112	73·4
912	155·7	99·3	136/96	109	147	59·3
840	124·0	106·3	123/8	100	166	48·1
Mean	**146·8**	**121·4**	**139/106**	**117**	**146**	**65·0**
± SE	±5·8	±9·9	8·4/8·3	±8·2	±9·0	±5·0

LEGEND.

H. R. = Heart Rate (b/min)
$_{sa}$BP = Systemic Mean Blood Pressure (mmHg)
$_{m}$BP = Mesenteric Blood Pressure (mmHg)
$_{\bar{m}}$BP = Mesenteric Mean Blood Pressure (mmHg)
F = Mesenteric Flow (ml/min)
R = Mesenteric Resistance (Kdyne/s/cm^{5})

Stimulus induced ^{3}H-noradrenaline overflow results.

1. Untreated male Wistar rats.

	control DPM	overflow DPM	difference DPM
	2277·6	4047·6	1769·9
	3367·5	4498·3	1130·8
	3367·5	4377·7	1269·4
	3479·4	4526·4	1047·0
	3024·5	3834·5	810·0
mean	**3051·5**	**4256·9**	**1205·4**
SE	±188·2	±121·3	±142·8

2. Male Wistar rats pretreated for 21 days with atenolol.

	control DPM	overflow DPM	difference DPM
	5030·1	6270·1	1240·0
	3901·8	4295·1	393·3
	2799·1	3181·9	382·8
	1986·0	2216·7	230·7
	3719·1	4434·5	715·4
mean	**3487·2**	**4079·7**	**592·4**
SE	±461·9	±608·2	±161·1

PUBLICATIONS.

In order of inclusion in the appendix:

Kingsbury, M. P., Draper, A. J., Refern, P. H. & Todd, M. H.
Chronic effects of atenolol in spontaneously hypertensive rats.
Br. J. Pharmac., 1988, **94**, 419P

Kingsbury, M. P., Draper, A. J., Refern, P. H. & Todd, M. H.
Chronic effects of atenolol in Wistar rats.
Br. J. Pharmac., 1988, **94**, 420P

Kingsbury, M. P., Draper, A. J., Refern, P. H. & Todd, M. H.
Chronic effects of atenolol on mesenteric vascular responses in dogs.
Br. J. Pharmac., 1988, **95**, 703P

Kingsbury, M. P., Draper, A. J., Refern, P. H. & Todd, M. H.
Vascular responses to atenolol and nitrendipine in the normotensive rat: evidence of a significant drug interaction.
Br. J. Pharmac., in press.

Kingsbury, M. P., Draper, A. J., Refern, P. H. & Todd, M. H.
Interaction between atenolol and nitrendipine in the hypertensive rat.
Br. J. Pharmac., in press.

CHRONIC EFFECTS OF ATENOLOL IN SPONTANEOUSLY HYPERTENSIVE RATS.

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Previous work has indicated that following chronic atenolol administration, changes in end-organ response are accompanied by altered presynaptic mechanisms. (Carr *et al*, 1983, Draper *et al*, 1986) The present study investigates this phenomenon in the spontaneously hypertensive rat (SHR).

Atenolol (50mg/kg) was administered orally in 5% polyethylene glycol (PEG) to male SHR's (300-350g); control results were obtained from animals dosed with vehicle alone. Blood pressure was measured in the conscious rat by the "tail-cuff" technique using the schedule described previously. (Kingsbury *et al*, this meeting) Twenty-four hours after the last dose of atenolol or PEG the *in situ* blood-perfused mesentery was prepared according to the method of Jackson and Campbell (1980). Vasoconstricor responses to exogenous noradrenaline and periarterial electrical stimulation were assessed as previously described. (Kingsbury *et al*, this meeting)

After the first day of dosing with atenolol, blood pressure in the conscious rat was significantly ($p<0.001$,n=4) reduced from 214.5 ± 3.4mmHg in control animals to 165.3 ± 5.2mmHg in atenolol-treated animals. After 5 days the mean blood pressure of the control (PEG), group was 227 ± 3.2mmHg while that of the atenolol-treated group was 165.3 ± 5.2mmHg. This reduction was maintained throughout the dosing period; after 19 days atenolol-treated animals had a mean blood pressure of 185.3 ± 3.7mmHg compared to 226 ± 5.1 in controls. Results from the *in situ* mesentery showed that treatment with atenolol for 7 days resulted in increased vasoconstriction in response to higher doses of noradrenaline. After 21 days this potentiation had disappeared, and was replaced by a significant ($p<0.001$,n=7) reduction in response to lower doses of noradrenaline. Response to periarterial electrical stimulation was unchanged after 7 days atenolol treatment. This was in contrast to the highly significant ($p<0.001$) decrease in response after 21 days atenolol treatment. (fig 1)

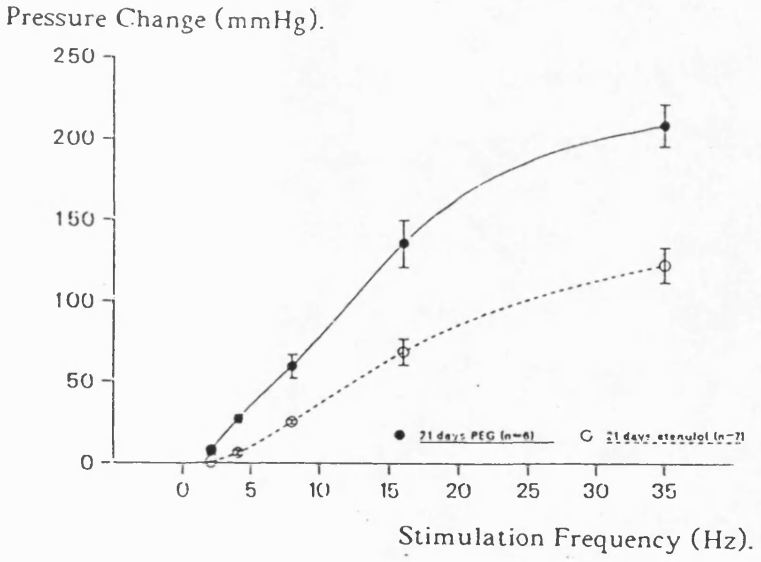

Fig 1: Effect of 21 days Atenolol p.o. on Electrical Stimulation.

These results indicate that chronic administration of atenolol caused a reduction in the blood pressure in the conscious SHR which reached a maximum after 3 days, and was maintained for at least 18 days thereafter. Although this reduction was highly significant, blood pressure in the SHR was still higher than in untreated normotensive animals. Results from the *in situ* mesentery indicate that after 21 days atenolol administration, there is a significant reduction in transmitter release from the presynaptic terminal, as evidenced by the reduced response to periarterial electrical stimulation. The time course and size of this effect suggests that it may be important in the antihypertensive effect of atenolol observed clinically.

Draper, A.J., Kendall, H.E. & Redfern, P.H. (1986) J. Auton. Pharmac. 5 259-268
Carr, S.R., Draper, A.J., Lamaa, M. & Redfern, P.H. (1983) J. Auton. Pharmac. 3, 7-12.
Jackson, E.K. & Campbell W.B. (1980) Eur. J. Pharmac. 66 217-224.
Kingsbury, M.P., Draper, A.J., Redfern, P.H. & Todd, M.H. (1988) this meeting

CHRONIC EFFECTS OF ATENOLOL IN WISTAR RATS.

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We have previously shown that after long-term administration of β-adrenoceptor blocking drugs in the rat, complex changes are produced quite distinct from the blockade of post-synaptic β-adrenoceptors. (Carr *et al*, 1983, Draper *et al*, 1986) These effects are more consistent with the time course of the antihypertensive action of β-adrenoceptor blocking drugs. The present study investigates further the effect of chronic β-adrenoceptor blockade on adrenergic neurotransmission using an *in situ* preparation to avoid complications arising from possible "wash-out" of antagonist following tissue removal.

Atenolol (50mg/kg) was administered daily p.o. to normotensive male Wistar rats (300-350g) in 5% polyethylene glycol (PEG). Control results were obtained from animals dosed with vehicle alone. Blood pressure was measured in the conscious rat by the "tail-cuff" technique and was recorded daily before and 2h after dosing. Twenty-four hours after the last dose of atenolol or PEG the degree of blockade was assesed by constructing dose response curves to isoprenaline injected as a bolus i.v. The *in situ* blood-perfused mesentery was prepared according to Jackson and Campbell (1980). Vasoconstrictor responses to exogenous noradrenaline (20-2000ng) and periarterial electrical stimulation (15v rectangular pulses of 1ms duration for 20sec. 2-35Hz) were obtained 24h after the last dose.

During the first week of dosing with atenolol, blood pressure in the conscious rat was significantly reduced. After 5 days the mean blood pressure of the control (PEG), group was 153±2.9mmHg while that of the atenolol treated group was 128.5±2.8mmHg ($p<0.001$,n=8). This reduction was maintained throughout the dosing period; after 19 days, atenolol-treated animals had a mean blood pressure of 130.1±2.0mmHg compared to 151.8±1.7mmHg in controls. ($p<0.001$)

	ISOPRENALINE (μg)						
	.0025	.005	.01	.02	.05	.1	.2
7d PEG	3.8±2.5	8.9±2.8	20±2.5	28.8±7.6	88.8±13.6	110±11.2	140±12.2
7d atenolol	2.5±2.2	8.8±5.1	25±7.5	30±10.6	50±18.1	60±15.8*	70±15**
21d PEG	0	3.1±1.5	18.9±5.9	35.6±9.9	88.1±4.8	118.1±4.3	158.8±8
21d atenolol	0	7.5±4.1	17.5±7.4	30±8.8	35±7.7***	55.0±8.3***	58.8±6.7***

Table 1 :- Mean Increase in Heart Rate (beats/min)±SEM, n=4 atenolol, n=8 PEG. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$

Results from the *in situ* mesentery showed that treatment with atenolol for 7 days resulted in increased vasoconstriction in response to higher doses of noradrenaline. This trend increased after 21 days treatment. In contrast, responses to higher frequencies of periarterial electrical stimulation were reduced after 21 days treatment with atenolol.

These results indicate that chronic administration of atenolol caused a reduction in blood pressure which reached a maximum after five days, and was maintained for at least 16 days thereafter. β-adrenoceptor blockade was established within 7 days and was maintained up to 21 days of dosing with atenolol (Table 1). Results obtained from the *in situ* mesentery further suggest that atenolol exerts a presynaptic action apparent after 21 days but not after 7 days drug treatment.

Draper, A.J., Kendall, H.E. & Redfern, P.H. (1986) J. Auton. Pharmac. 5 259-268
Carr, S.R., Draper, A.J., Lamaa, M. & Redfern, P.H. (1983) J. Auton. Pharmac. 3, 7-12.
Jackson, E.K. & Campbell W.B. (1980) Eur. J. Pharmac. 66 217-224.

CHRONIC EFFECTS OF ATENOLOL ON MESENTERIC VASCULAR RESPONSES IN DOGS.

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We have previously shown that, in the rat, chronic administration of β-adrenoceptor blocking drugs produces changes distinct from the post-synaptic blockade of β-adrenoceptors. (Carr *et al*, 1983, Draper *et al*, 1986) There is some evidence to suggest that atenolol may be acting presynaptically after chronic administration to normotensive and spontaneously hypertensive rats. (Kingsbury *et al*, 1988) These effects are consistent with the time course of the antihypertensive action of the β-blocking drugs. This study investigates the effect of chronic β-adrenoceptor blockade on adrenergic neurotransmission in normotensive dogs.

Atenolol (7.5mg/kg) was administered orally to male Alderley Park beagles (17-20kg) daily for 21 days (n=5). The animals were then anaesthatised with sodium pentobarbitone (30mg/kg i.v.) and maintained with a 1mg/min infusion. A laporotomy was performed and the superior mesenteric artery was cleared to allow the location of a doppler flow probe. Bipolar platinum electrodes were placed around the vessel distal to the probe; a suitable side-branch was cannulated to allow administration of drugs and measurement of mesenteric blood pressure. Following a 30min stabilisation period vasoconstrictor responses to exogenous noradrenaline (1-400ng) and periarterial electrical stimulation were obtained. Control results were obtained from untreated animals (n=6). Resistance was calculated using : $R\,(dyns/sec/cm^5) = \frac{mean\ blood\ pressure\ x\ 1330}{flow\ (mls/sec)}$ from mesenteric blood pressure and flow which were measured directly.

Changes in mesenteric pressure, flow and resistance to exogenous noradrenaline were not significantly different in control and drug treated animals. While there was some indication of a reduction following atenolol pretreatment in the response to electrical stimulation in terms of flow, this did not reach statistical significance. There was some evidence of a reduction in the mesenteric pressure response to electrical stimulation following atenolol pretreatment. However, when this data was used to calculate resistance a significant decrease in the mesenteric resistance response to electrical stimulation was observed. ($p<0.05$)

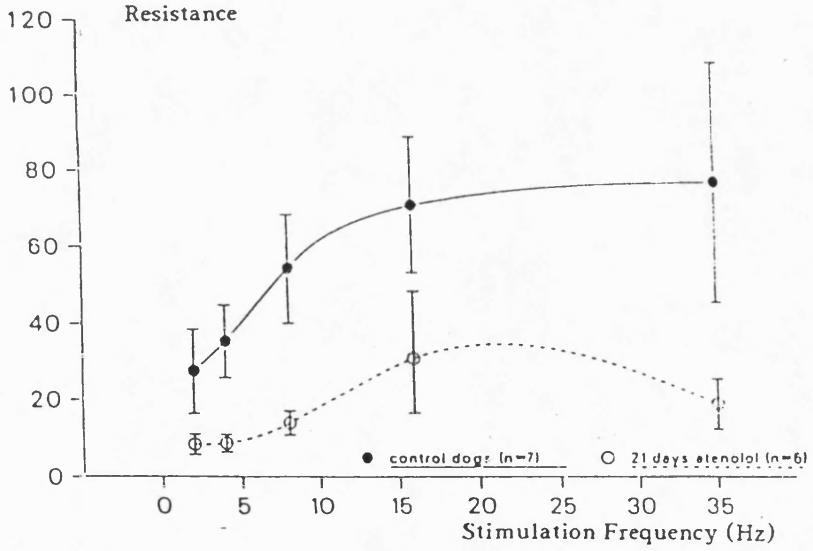

Fig 1: Effect of 21 days atenolol p.o. on resistance response to electrical stimulation

In conclusion, it seems likely that as neither mesenteric flow or pressure was kept constant during the experiment, animals were varying one or both of these parameters in response to stimulation. Only by using both these responses to calculate the response in terms of resistance did the overall trend become apparent. As chronic atenolol treatment produced no change in the response to exogenous noradrenaline but reduced the response to electrical stimulation, it is possible that, in accordance with our previous work in rats, it may be having some presynaptic effect.

Draper, A.J., Kendall, H.E. & Redfern, P.H. (1986) J. Auton. Pharmac. 5 259-268
Carr, S.R., Draper, A.J., Lamaa, M. & Redfern, P.H. (1983) J. Auton. Pharmac. 3, 7-12.
Kingsbury, M.P., Draper, A.J., Redfern, P.H. & Todd, M.H. (1988) Br. J. Pharmac. in press.

Vascular responses to atenolol and nitrendepine in the normotensive rat: evidence of a significant drug interaction.

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The β-adrenoceptor blocking drugs and the calcium antagonists are both established as effective antihypertensive agents, and they are increasingly used in combination. We have previously shown (Kingsbury et al 1988) that, after chronic administration to normotensive rats, the β-adrenoceptor blocking drug atenolol produces a significant reduction in the vasoconstrictor response to sympathetic nerve stimulation. This effect is in addition to the blockade of post-synaptic β-adrenoceptors which is established in the first few days of treatment, and is apparently caused by a reduction in transmitter release. In a similar series of experiments we have examined the effect of the calcium antagonist nitrendipine, alone and in combination with atenolol, on blood pressure and vascular reactivity.

Atenolol 50mg kg^{-1} and nitrendipine 3mg kg^{-1} were orally administered alone or in combination to groups of male Wistar rats (300-350g). The drugs were administered in polyethylene glycol, and control groups received vehicle alone. Blood pressure was monitored daily in the conscious animal by the tail-cuff method before and 2-hours after dosing. After drug administration for 21 days the responses to exogenous noradrenaline and to periarterial electrical stimulation were measured in the in situ blood perfused mesentery, (Jackson and Campbell 1980). The tissue was perfused at a constant rate and increased vascular resistance was measured as an increase in perfusion pressure.

Treatment with nitrendipine alone did not significantly alter the dose response curve to noradrenaline, but slightly decreased the response of the tissue to nerve stimulation over the range of frequencies used (2 - 35 Hz). This finding is consistent with the relatively small hypotensive effect of nitrendipine in these animals and with the suggestion that the calcium antagonists lower blood pressure effectively only when peripheral resistance is raised. After treatment for 21 days with the combination of drugs, vascular responses to noradrenaline were slightly but significantly decreased; in contrast, periarterial nerve stimulation now produced a significantly enhanced response. The responses to electrical stimulation are summarized in Table 1. Thus while treatment with either atenolol or nitrendipine alone resulted in a significant decrease in response to nerve stimulation, chronic administration of the two drugs in combination reversed this response. The mechanism responsible for this interaction is at present unknown. It may be suggested that the two drugs together produce a reduction in vascular reactivity sufficiently profound as to trigger some compensatory change in presynaptic mechanisms - for instance an enhanced release of a vasoconstrictor peptide co-released with noradrenaline. Whatever the mechanism, it may account for the failure to observe any additive hypotensive effect of atenolol and nitrendipine in these experiments.

Table 1 Increase in mesenteric perfusion pressure (mm Hg) after periarterial stimulation

Frequency (Hz)	2	4	8	16	35
Control	7.4±1.2	14.6±1.9	30.6±2.7	62.4±4.8	119.6±3.9
Nitrendipine	1.5±1.2	5.9±1.4	14.9±2.8	37.9±9.2	67.1±18.3
Combination	7.8±1.7	17.5±2.5	33.3±4.8	100.4±8.9	185.3±11.7

Kingsbury, M.P., Draper, A.J. et al, (1988) Br. J. Pharmac. 94: 420P,
Jackson, E.K., Campbell, W.B., (1980) Eur. J. Pharmac. 66: 217-224

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We have previously demonstrated a significant degree of interaction between the β-adrenoceptor blocking drug atenolol and the calcium antagonist nitrendipine in terms of vascular reactivity after chronic administration to normotensive rats, (Draper et al this meeting). Both classes of drug are more effectively depressor, and produce a more profound decrease in vascular reactivity when blood pressure and peripheral resistance are elevated; it was therefore of interest to examine the effects of these two drugs in hypertensive animals.

The design of the experiment and the methods used were exactly those previously employed, (Draper et al 1989), except that the animals were spontaneously hypertensive rats of the Japanese Okamoto strain, weighing approximately 200g. With all three regimens - atenolol 50mg kg^{-1} alone, nitrendipine 3mg kg^{-1} alone, and the two drugs together, - blood pressure fell rapidly during the first week and was constant thereafter. As in normotensive animals, the fall in blood pressure caused by the combination was not significantly greater than that produced by either drug alone.

Vascular responses were measured after 7 and 21 days drug treatment in the blood perfused *in situ* mesentery preparation, for clarity, only the results obtained after 21 days will be described here. The vasoconstriction produced by exogenous noradrenaline was significantly reduced by all three treatments and, as would be predicted, the effect was much greater than that seen in normotensive animals. In both the atenolol-treated and nitrendipine-treated groups, the response to periarterial nerve stimulation was significantly reduced compared to that seen in control preparations. In contrast, only a slight reduction, which did not reach statistical significance, was observed in animals treated with the combination.

Table 1 Increase in mesenteric perfusion pressure (mg Hg) after periarterial stimulation

Frequency (Hz)	2	4	8	16	35
Control	7.8±3.3	26.7±2.8	59.5±7.5	135.0±14.5	208.3±12.8
Nitrendipine	5.7±0.4	13.2±1.8	28.2±4.4	59.5±12.3	117.5±12.2
Combination	8.0±1.2	20.0±2.9	41.6±6.8	100.4±12.5	189±17.1

Thus the overall picture is the same in normotensive and hypertensive animals in that the decrease observed after either drug alone is significantly reduced after combined drug treatment. The difference in the magnitude of the interaction can probably be accounted for by the enhanced depressor responses obtained in the hypertensive animals. Thus we have demonstrated that in hypertensive as well as normotensive rats there is clear evidence of a significant degree of interaction between atenolol and nitrendipine, leading to a smaller reduction in blood pressure than might be expected. Whether this interaction can also occur in man remains to be seen.